Shedding of the „capsule“ and proteinase activity in the horse embryo

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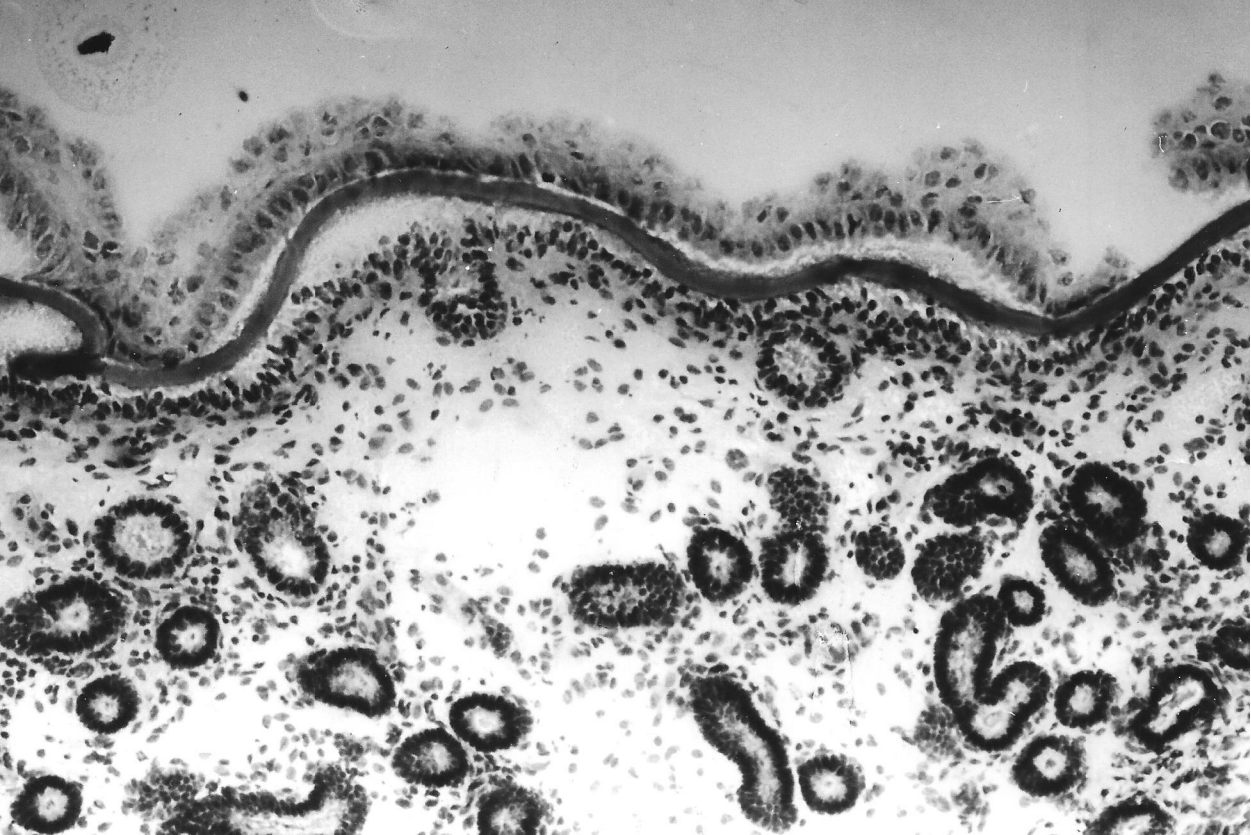
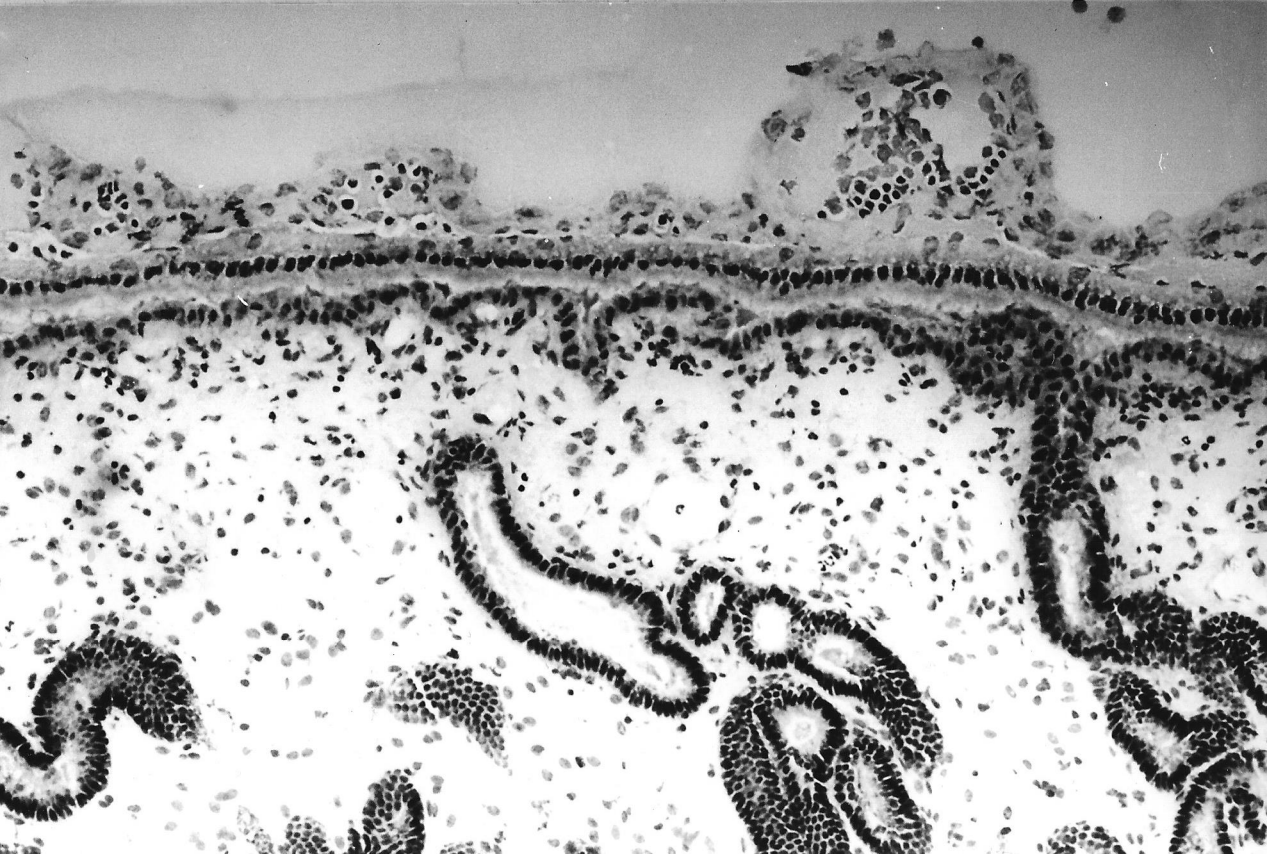
ABSTRACT

To establish cellular contact with the endometrium, the horse conceptus must first shed a glycoprotein "capsule". To investigate whether this process involves a proteinase system analogous to that found in rabbits, the uteri from 5 mares have been studied. They were collected at surgery or slaughter on Days (D) 20.5, 21.5 and 28.5 after diagnosed ovulation, or on D 28 and D 30 as estimated from conceptus diameters measured by ultrasonography. Native cryostat sections were studied with the highly sensitive histochemical gelatin film test for localisation of proteinase using a series of inhibitors for their identification. At D 20.5 the capsule was still complete; at D 21.5 it was still well preserved although completeness could not be ascertained since the specimen had collapsed; at D 28 and after, only minor possible remnants were seen. Proteinase activity was considerable in the embryonic membranes and the adjacent endometrium but much lower in the conceptus-free horn. Two categories of proteinases could be identified: one with acid pH optimum (cathepsin-like) in the uterine epithelium and embryonic membranes; another with alkaline pH optimum in portions of the trophoblast at D 20.5 and 21.5. The possible role of the latter enzyme in dissolution of the capsule requires further study of D 21 - 28 specimens. Supported by NSERC

MORPHOLOGY

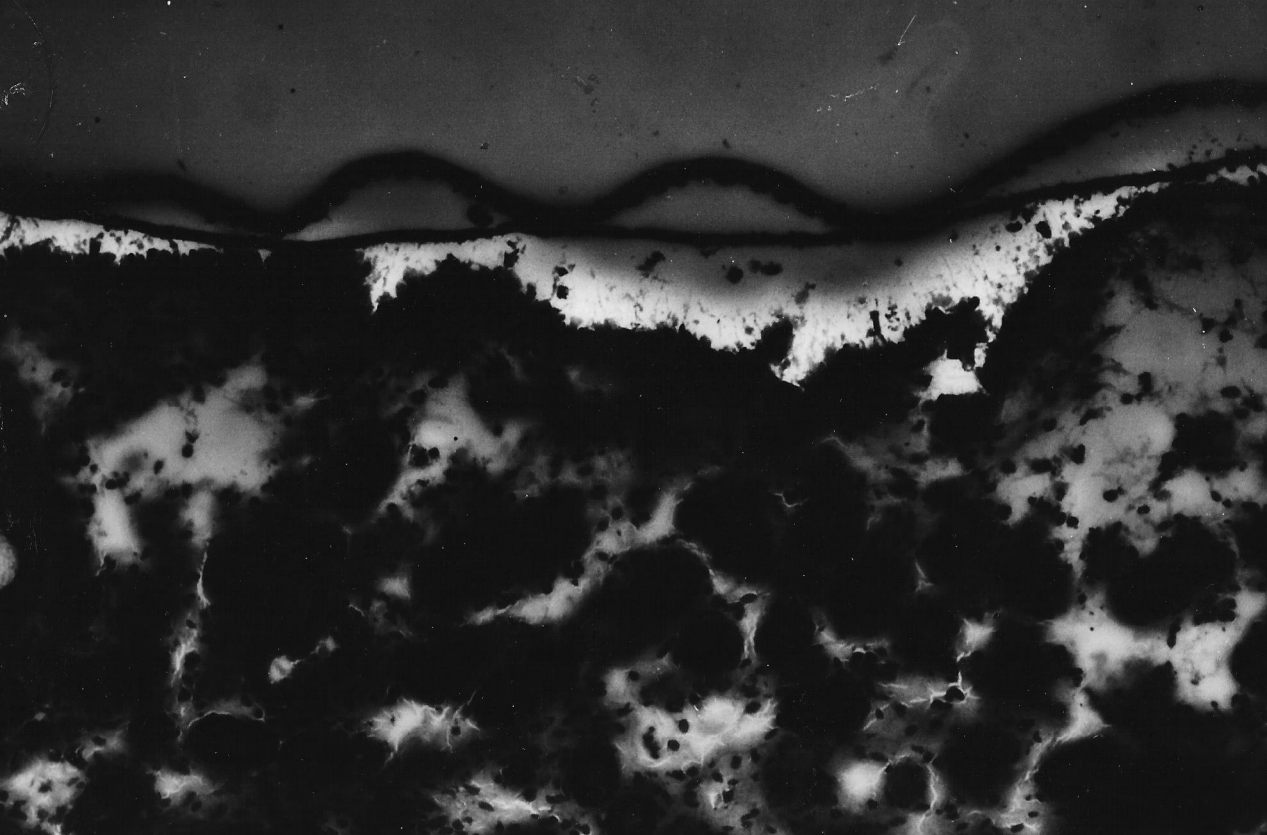


Morphology, D 20.5, fetal membranes and endometrium, cryostat section, toluidine blue, x 200. The "capsule" is still intact at this stage and can be easily identified as an intensely stained band-like structure interposed between the fetal membranes (here: trilaminar yolk sac wall) and the uterine epithelium. This holds true for the entire surface of the conceptus.

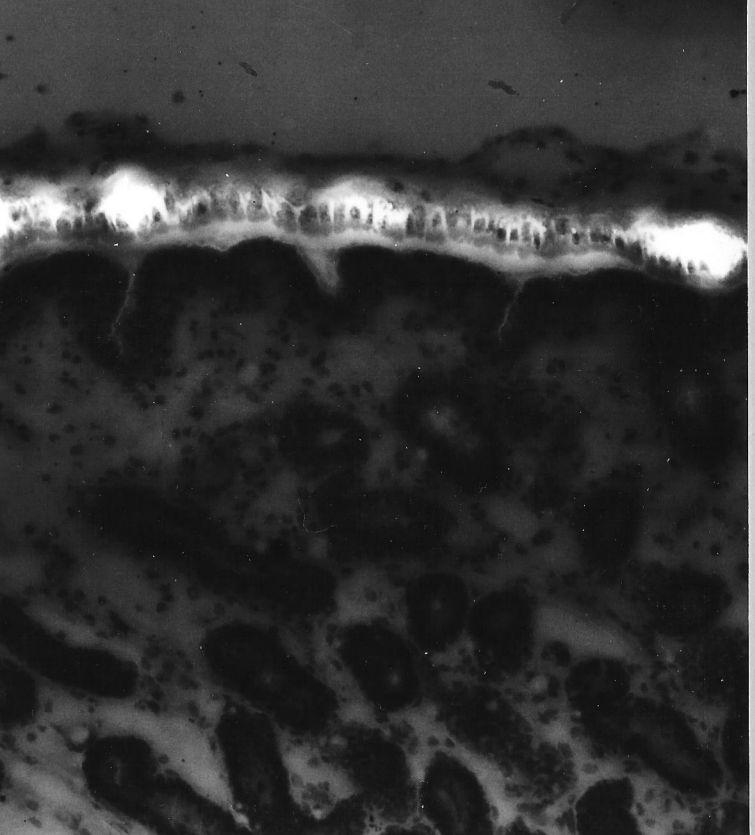


Morphology, D 28.5, fetal membranes and endometrium, cryostat section, toluidine blue, x 200. The "capsule" has disappeared from all areas of the fetal membranes, and only a very narrow gap remains between the trophoblast of the trilaminar yolk sac wall and the uterine epithelium.

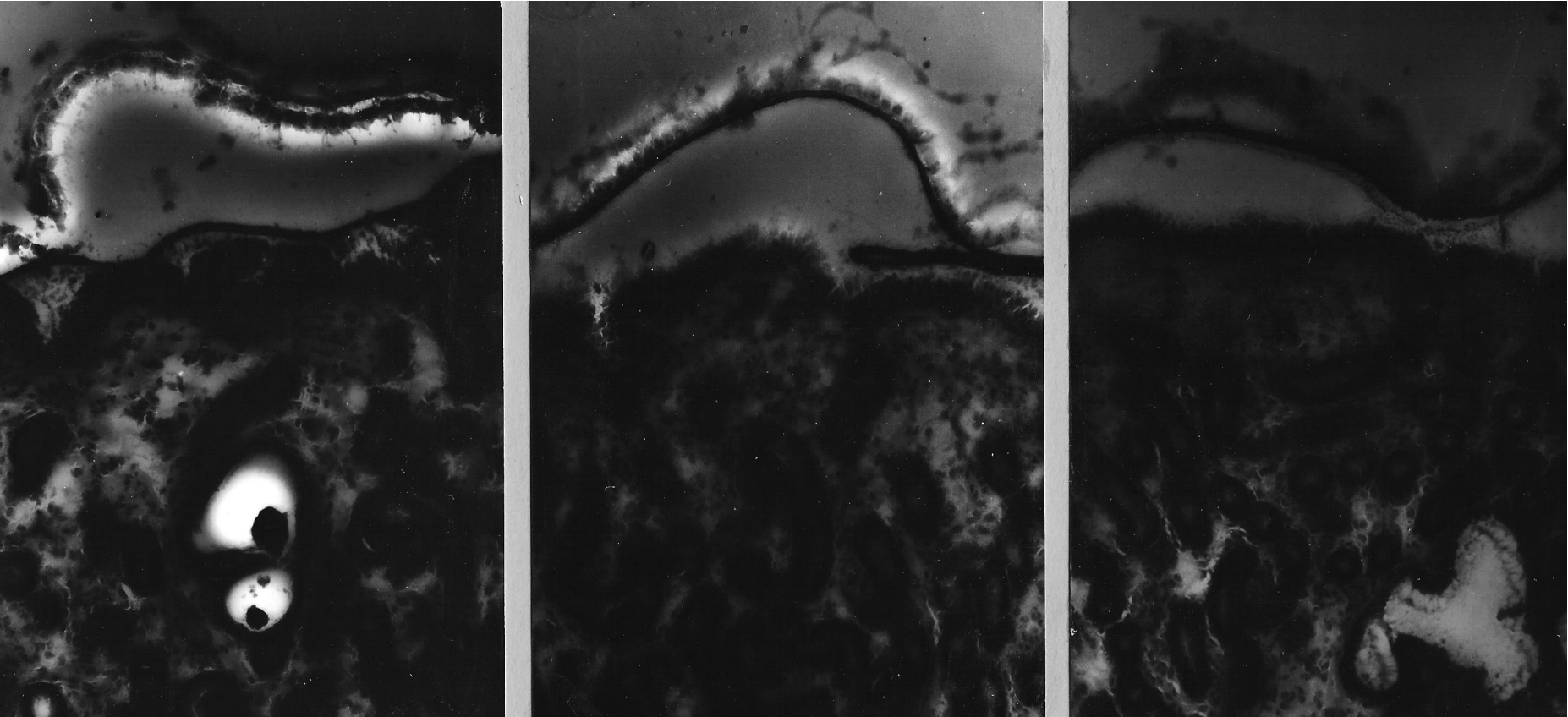
HISTOCHEMISTRY



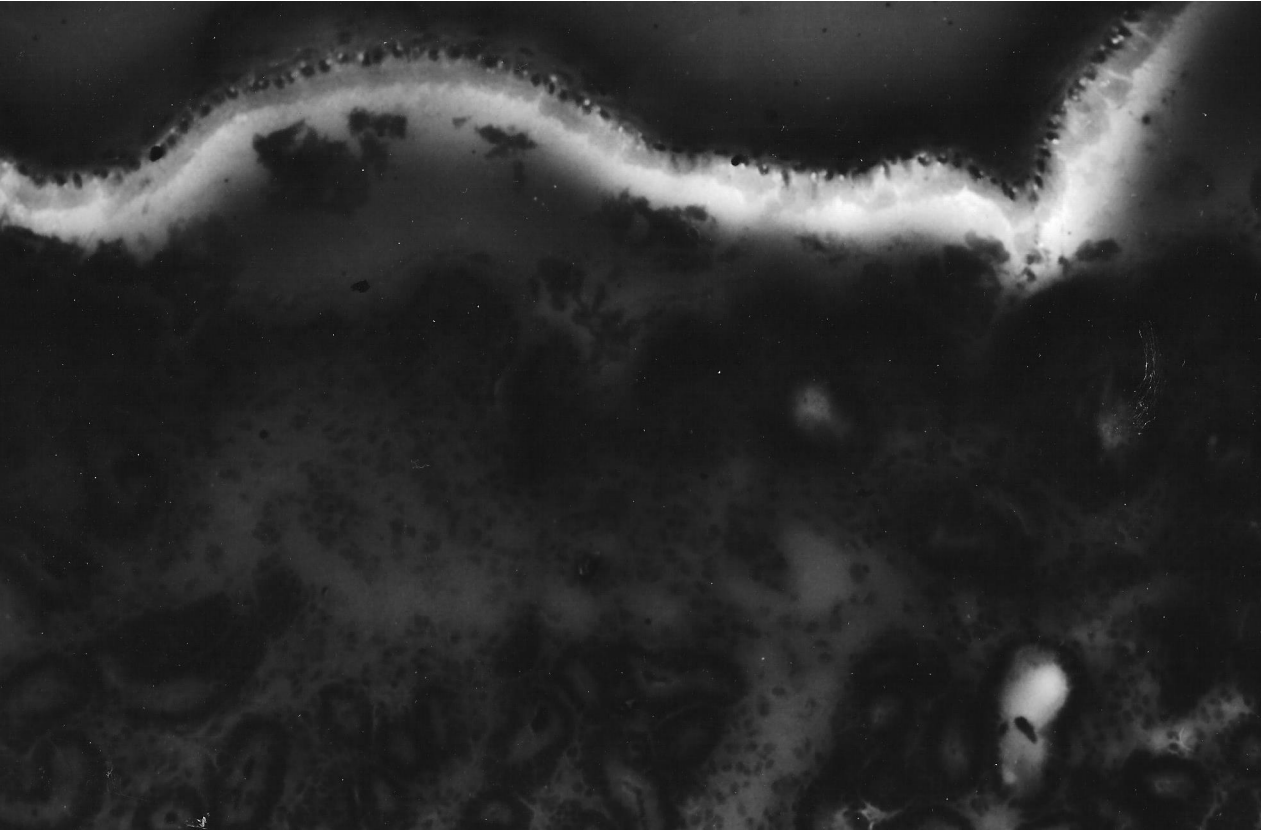
Gelatin film test, D 20.5, pH 5, incubation period 15 min, x 200. Bright areas on the dark background indicate considerable activity of acid proteinase at the uterine luminal epithelium (note short incubation period). Bilaminar yolk sac wall (above) negative. "Capsule" (straight dark line) easily recognizable.



Gelatin film test, D 28.5, pH 5, incubation period 15 (left) or 60 (right) min., x 200. The acid proteinase activity of fetal membranes is maximal at the basal pole of trophoblast cells (left); it is so high that a very broad lysis zone, which is symmetrically located at both sides of the fetal membrane, results after 60 min. incubation (right).



Gelatin film test, D 20.5, pH 8, incubation period 60 min., x 140. Left: Control (no inhibitor). Alkaline proteinase activity found in the trophoblast (here of the trilaminar yolk sac wall) and in some widened lumina of endometrial glands. "Capsule" follows the surface of the uterine epithelium, while fetal membranes have artificially detached. Middle: Soybean trypsin inhibitor blocks activity in endometrial glands (serine endopeptidase) but not in the trophoblast. "Capsule" follows in this case the fetal membranes. Right: Antipain inhibits trophoblast proteinase as well as the enzyme of endometrial glands.



Gelatin film test, D 28.5, pH 8, incubation period 60 min., x 200. The proteinase of the trophoblast shown here is somewhat difficult to discriminate from the acid proteinase activity which is very high at this stage (see previous picture) and which may not have been suppressed completely by the alkaline buffer. Discrimination is more clear-cut at D 20.5 (see above). Both enzymes are characterized as thiol endopeptidases on the basis of inhibitor experiments (see Tables). Note, however, that alkaline proteinase is maximal at the apical part of trophoblast cells. Activity in glandular lumen is due to an alkaline serine proteinase as judged from inhibitor experiments (see Tables).

Characterization of proteinases of conceptus and endometrium of the horse by interaction with various proteinase inhibitors

(Gelatin substrate film test, methodology see Denker, 1977; stages studied: days 20.5 and 28.5)
(+: inhibition; Ø : no effect; ↑: activation)

Inhibitor	Acid proteinase (pH 5) (*)	Alkaline proteinase (pH 8)	
		Trophoblast	Glandular lumina
Soybean trypsin inhibitor	Ø	Ø	+
Aprotinin (Trasylol)	Ø	Ø	+
Antipain	+	+	+
Iodoacetamide	+	+	Ø
E-64	+	+	Ø
EDTA+Dithiothreitol	↑	↑	Ø
Phosphoramidon	Ø	Ø	Ø

*: Fetal membranes (D 28.5 > D 20.5); uterine luminal epithelium

Assignment of proteinases to catalytic classes

	Class of proteinase			
	Serine	Thiol	Carboxyl	Metallo
Typical pH	7 - 9	4 - 7	2 - 5	7 - 9
Soybean trypsin inhibitor	+	Ø	Ø	Ø
Aprotinin (Trasylol)	+	Ø	Ø	Ø
Antipain	+	+	Ø	Ø
Iodoacetamide	Ø	+	Ø	(Ø)
E-64	Ø	+	Ø	Ø
EDTA+Dithiothreitol	Ø	↑	Ø	+
Phosphoramidon	Ø	Ø	Ø	+

CONCLUSION

Inhibition profiles allow to identify 3 main gelatinolytic proteinases in horse fetal membranes and uterus:

1. an acid thiol proteinase (cathepsin) predominating in the trilaminar yolk sac wall (D 28.5 > D 20.5) and in the uterine luminal epithelium;
2. an alkaline thiol proteinase in the trophoblast (most easily identified at D 20.5);
3. an alkaline serine proteinase in endometrial glandular lumina (uterine secretion).

More detailed studies including additional material are needed in order to establish correlations of proteinase activity levels with the various regions of the fetal membranes and with the process of dissolution of the "capsule", focussing on stages between D 20 and 28.

REFERENCES

Betteridge, K.J., Eaglesome, M.D., Mitchell, D., Flood, P.F., Beriault, R.: Development of horse embryos up to twenty two days after ovulation: observations on fresh specimens. J.Anat. 135, 191-209 (1982)

Denker, H.-W.: Protease substrate film test. Histochemistry 38, 331-338 and 39, 193 (1974)

Denker, H.-W.: Interaction of proteinase inhibitors with blastocyst proteinase involved in implantation. In: Protides of the Biological Fluids (H. Peeters, ed.) XXIII. Oxford: Pergamon Press, 1976, pp. 63-68

Denker, H.-W.: Implantation. The Role of Proteinases, and Blockage of Implantation by Proteinase Inhibitors. Berlin: Springer-Verlag, 1977 (Adv.Anat. Embryol.Cell Biol., Vol. 53, Part 5)

Flood P.F., Betteridge, K.J., Diocee, M.S.: Transmission electron microscopy of horse embryos 3-16 days after ovulation. J.Reprod.Fert. 32, 319-327 (1982)