Studies on the Egg Yolk Agglutinating Factors in Boar Semen

By Hiroshi MASUDA*

Animal Reproduction Division, National Institute of Animal Industry
(Yatabe, Ibaraki, 305 Japan)

The author and his co-workers have found out that, when the diluents including egg yolk for freezing of boar spermatozoa were used, egg yolk agglutinated immediately after the dilution and most of the spermatozoa were enclosed into the agglutinated masses and their motility was drastically suppressed. On the other hand, by using skim milk-diluent widely used for storing liquid boar semen at 15°C in the field, agglutination in diluted semen has seldom occurred. This agglutination of diluted boar semen has been a great disadvantage for practice of artificial insemination on swine.

The present experiment was performed to clarify the characteristics of factors causing agglutination, and to contribute to elongation of the survivals of boar spermatozoa in dilution, by preventing the occurrence of the agglutination.

Agglutination phenomena and the origin of agglutinating factors

The agglutinating factors inducing agglutination of egg yolk diluent were found in most of the semen from boars regardless of breeds, but not in bull, buck and cock semen. They were derived from the secretion of seminal vesicle and were absent in the secretion of the other accessory reproductive glands.

By Ouchterlony's agar gel diffusion method, agglutination positive boar semen developed precipitation line against egg yolk, sera and sometimes against milk. This precipitation phenomena was proven to be a different reaction superficially resembling true antigen-antibody reactions in another experiment.

* Present address: Tohoku National Agricultural Experiment Station (Shimo-kuriyagawa, Morioka, Iwate, 020-01 Japan)

Variations in the activity of agglutinating factors

There were many variations in the activity of egg yolk agglutinating factors of semen between boars. From the results of averaging of 153 samples obtained through out the year, concentrations of protein, citric acid and zinc in seminal plasma having high agglutinating activity showed a tendency to be higher than those in seminal plasma having low agglutinating activity.

The agglutinating activity began to rise around the end of summer and maintained high level to the end of autumn, then decreased in winter and/or spring season. The concentrations of protein, citric acid and zinc in seminal plasma varied in almost parallel with the variation of the agglutinating activity. These findings indicated that the agglutinating activity was closely related to the function of accessory reproductive organs, especially seminal vesicles.

The interval of semen collection did not affect on the agglutinating activity.

The association of agglutinating activity with some physical properties and chemical constituents in boar seminal plasma

There was no marked difference in general

Table 1. Concentrations of chemical components in boar seminal plasma having low protein agglutinating activity and having high agglutinating activity (N=20—22)

Components	Agglutinating activity		Mean±S.D.
	Below 0.2	Above 1	
Protein-N	336.3 ±138.6	615.7* ±113.8	472.7±189.1
Citric acid	69.9 ± 37.7	154.8**± 28.9	113.3± 54.3
Cl	389.4 ± 92.5	359.5 ± 35.5	373.7± 69.6
Na	384.3*±135.2	313.0 ± 76.3	348.7±114.3
K	62.5 ± 27.2	59.6 ± 15.9	60.2± 21.5
Mg	6.4 ± 4.8	10.1* ± 0.9	8.5± 3.8
Ca	1.8 ± 0.7	2.6* ± 0.8	2.3± 0.8
Zn	1.2 ± 0.6	5.7**± 2.0	3.4± 2.7

Note: The agglutinating activity was expressed as optical density at 550 nm of reaction mixture of 1 part of boar seminal plasma and 9 parts of 10% egg yolk solution. * $p < 0.05$, ** $p < 0.001$

properties of the semen depending on the difference of the agglutinating activities.

Osmotic pressure of the seminal plasma having high agglutinating activity was higher than that of the seminal plasma having low agglutinating activity, but the electric conductivity of the former was significantly high as compared with that of the latter. This data suggested that the semen having high agglutinating activity was in the condition of lower ionic strength.

Table 1 shows the concentration of seminal constituents as related to different agglutinating activity. The semen having high agglutinating activity was characterized by high concentration of protein, citric acid and zinc and by low concentration of sodium.

Properties of agglutinating factors and influential conditions for agglutinations

The agglutinating activity was affected by egg yolk level, pH value, ionic strength of the diluent and incubation temperature. The most active agglutination occurred on 10% level of egg yolk and pH about 7 at the range from 30° to 50°C. Above 30% of egg yolk the agglutinating activity was inhibited.

Agglutination of egg yolk solution with boar seminal plasma was initiated immediately after dilution and reached the plateau at 30°C about 60 minutes later. The pH value of egg yolk solution changed little during incubation. The agglutinating activity was also inhibited in the presence of high concentrations of various kinds of chlorides.

These findings shows that high ionic strength is useful to prevent agglutination.

Purification of egg yolk agglutinating factors

Boursnell and Coombs revealed a haemagglutinin in boar seminal plasma originating in the vesicular secretion. This haemagglutinin was eluted between peaks A and B by gel filtration method. Nelson and Boursnell isolated 7 haemagglutinins, which had different activities themselves towards the red blood cells of different animal species, from boar seminal plasma by using Amberite XE-64.

The author tried to purify the egg yolk agglutinating factors by several methods. By means of gel filtration of boar seminal plasma with Sephadex G-100 using tris-buffer added 1M-NaCl and acetate buffer, the proteins of the boar seminal plasma were separated into four to five peaks.

Considerably purified agglutinating factors were obtained at the second peaks by the gel filtration starting from the agglutinated substances obtained from the mixture of 3 parts of 10% egg yolk solution and 1 part of boar seminal plasma.

With starch gel electrophoresis using formic

acid-acetic acid buffer, purified agglutinating factors were separated into three zones consisting six to seven components. Since each extracted components from these three zones reacted sharply with egg yolk solution in agar gel, it may be concluded that the agglutinating activities reside in group of proteins. In further experiment the agglutinating activity was absorbed by the red blood cells of various animal species.

Since these findings showed that egg yolk agglutinating factors were closely similar to the haemagglutinin, their identity was postulated.

Some constituents of egg yolk caught by the agglutinating factors

Figure 1 shows the electropholetic patterns of agglutinated substances in the mixture of egg yolk solution and boar seminal plasma, and some egg yolk proteins using starch-urea gel electrophoresis with a formic-acetic acid buffer at pH 1.7. At this pH, all the proteins migrated to the cathode. Agglutinated substances of egg yolk and seminal plasma were resolved into seven components. Components II–1 and 2 and IV–2 of agglutinated substances corresponded some parts of lipovitellin and

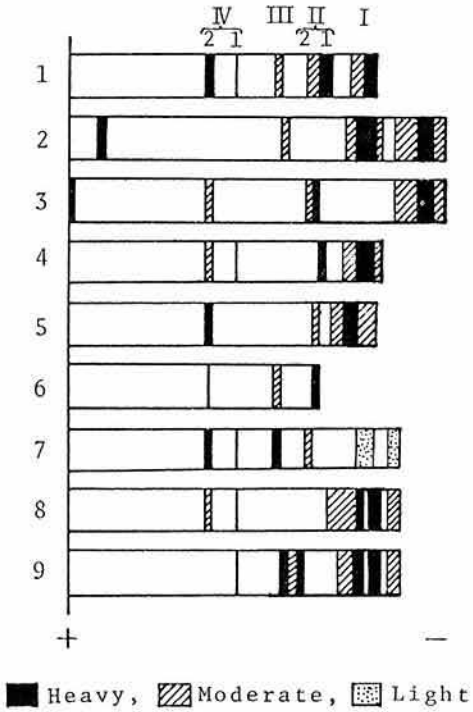

Fig. 1. Electropholetic patterns of agglutinated substances in the mixture of egg yolk solution and boar seminal plasma, and of some egg yolk proteins.
1. 10% egg yolk, 2. Boar seminal plasma, 3. Agglutinated portion, 4. Not agglutinated portion, 5. Lipovitellin, 6. Lipovitellenin, 7. Livetin I, 8. Livetin II, 9. Livetin III.

Table 2. Quantitative changes of egg yolk lipoprotein and neutral fats by agglutination

Sample No.	Agglutinating activity	Lipoprotein (mg/dl)			Neutral fat (mg/dl)		
		Before agglutination	Agglutinated lipoprotein	Rate of agglutination (%)	Before agglutination	Agglutinated neutral fat	Rate of agglutination (%)
1	0.020	120.0	0	0	558.3	60.4	10.8
2	0.023	120.0	0	0	558.3	43.7	7.8
3	0.025	120.0	0	0	558.3	43.7	7.8
4	0.120	138.1	26.1	18.9	732.0	56.0	7.6
5	0.580	149.8	78.4	55.3	732.0	582.0	79.5
6	0.810	182.8	138.1	75.5	790.0	742.0	93.9
7	0.870	130.6	56.0	42.9	714.0	424.0	59.4
8	0.880	149.3	93.3	62.5	824.0	810.0	98.3
9	0.949	160.5	115.7	72.1	790.0	768.0	97.2
10	1.160	116.4	87.3	75.0	637.5	554.2	86.9
11	1.200	149.3	74.6	50.0	714.0	470.0	65.8
12	1.240	132.8	92.8	69.9	558.3	520.8	93.2
13	1.260	116.4	83.7	71.9	579.2	537.5	92.8

lipovitellenin. These findings showed that some parts of lipovitellin and lipovitellenin were caught by the agglutinating factors.

Table 2 shows the quantitative changes of egg yolk lipoprotein and neutral fats by agglutination. About 64% (43–75%) of lipoprotein and about 85% (60–98%) of neutral fats in the mixture of egg yolk solution and boar seminal plasma having high agglutinating activity were precipitated by agglutination.

From these results obtained above, it was concluded that lipovitellin, lipovitellenin and neutral fats of egg yolk would be caught by the agglutinating factors in boar seminal plasma.

References

1) Masuda, H., Soejima, A. & Waide, Y.: Agglutination phenomena of various kinds of dilutors with boar semen and the origin of agglutinating factors in the semen. *Jap. J. Anim. Reprod.*, **23**, 121–125 (1977) [In Japanese, English summary].
2) Masuda, H., Soejima, A. & Abe, T.: Immunological studies on protein precipitating factors in boar semen. *Jap. J. Anim. Reprod.*, **23**, 142–147 (1977) [In Japanese, English summary].
3) Masuda, H., Soejima, A. & Waide, Y.: Variations in the activity of the protein agglutinating factors of boar semen owing to the conditions of semen collection. *Jap. J. Anim. Reprod.*, **25**, 194–197 (1979) [In Japanese, English summary].
4) Masuda, H., Soejima, A. & Waide, Y.: The association of protein agglutinating activity with some physical properties and chemical constituents in boar seminal plasma and secretions of accessory reproductive glands. *Jap. J. Anim. Reprod.*, **26**, 121–125 (1980) [In Japanese, English summary].
5) Masuda, H., Soejima, A. & Waide, Y.: Properties of protein agglutinating factors in boar semen and influential conditions for agglutination. *Jap. J. Anim. Reprod.*, **23**, 138–141 (1977) [In Japanese, English summary].
6) Masuda, H., Soejima, A. & Waide, Y.: Purification of the protein agglutinating factors in boar seminal plasma. *Jap. J. Anim. Reprod.*, **26**, 24–29 (1980) [In Japanese, Englush summary].
7) Boursnell, J. C. & Coombs, R. R. A.: A haemagglutinating factors in boar seminal plasma. *J. Reprod., Fert.*, **11**, 139–144 (1966).
8) Schellpfeffer, D. A. & Hunter, A. G.: Electrophoretic and gel filtration behaviour of boar seminal plasma proteins before and after removal of accessory sex glands. *J. Reprod. Fert.*, **23**, 291–298 (1970).
9) Nelson, M. & Boursnell, J. C.: Studies of boar seminal plasma proteins. IV. Isolation of factors with haemagglutinating and protein-precipitating activity. *Biochem. Biophys. Acta*, **117**, 144–156 (1966).
10) Masuda, H., Soejima, A. & Waide, Y.: Some constituents of egg yolk caught by the agglutinating factors in boar seminal plasma. *Jap. J. Anim. Reprod.*, **26**, 129–133 (1980) [In Japanese, English summary].

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