

ELECTROPHORETIC AND GEL FILTRATION BEHAVIOUR OF BOAR SEMINAL PLASMA PROTEINS BEFORE AND AFTER REMOVAL OF ACCESSORY SEX GLANDS

D. A. SCHELLPFEFFER AND A. G. HUNTER

*Department of Animal Science, University of Minnesota,
St Paul, Minnesota 55101, U.S.A.*

(Received 24th November 1969)

Summary. The origins of the major proteins of boar seminal plasma were identified using starch gel electrophoresis, gel filtration on Sephadex G-200 and surgical removal of various accessory sex glands.

Seminal vesicular secretion accounted for all the major proteins in seminal plasma which migrated to the cathode. Bulbo-urethral proteins could not be detected electrophoretically or by gel filtration. Prostatic-urethral fluid contained at least one protein which migrated to the anode and was not of serum origin. Secretions from the epididymis and/or testis contained at least two proteins which migrated to the anode that were eventually voided in the seminal plasma of the ejaculate.

Boar seminal plasma separated on Sephadex G-200 into three major peaks corresponding to molecular weights of approximately 155,000 (Peak B), 55,000 (Peak A) and 34,000 (Peak C). Peak A proteins were primarily of seminal vesicular origin, while Peak B proteins were primarily of epididymal or testicular origin. Peak C consisted of proteins from all regions of the reproductive tract. Haemagglutinating activity was located between Peaks A and B, associated with molecules of approximately 68,000 molecular weight, and was absent after removal of the seminal vesicles.

INTRODUCTION

Bournsnel, Johnson & Zamora (1962) reported that the electrophoretic properties of the major proteins of boar seminal plasma were similar to those of the vesicular secretion. Nelson & Bournsnel (1966), using gel filtration, reported that two proteins (Fractions A and B) constituted 98% of the total protein in boar seminal plasma. The iso-electric point of Protein A was 8.8 and of Protein B was 4.6 (Bournsnel & Briggs, 1969). Ultracentrifuge studies gave an $s_{20,w}$ value of 2.3 S for Protein A and 6.6 S for Protein B (Bournsnel & Briggs, 1969). A third protein was also isolated from vesicular secretions (Bournsnel & Coombs, 1966; Bournsnel, 1967). This protein, now designated Protein H, had haemagglutinating properties. Gel-filtration studies by Bournsnel, Briggs & Cole (1968)

revealed that this haemagglutinin occurred as a distinct peak masked by the greater protein content of Protein A. Electrophoretic studies by Bournsnel & Briggs (1969) showed that the haemagglutinating protein was positively charged and possessed an iso-electric point of about 9.4.

The objectives of this investigation were to achieve electrophoretic and chromatographic definition of the proteins in boar semen both before and after surgical removal of the accessory sex glands. Another objective was to determine the origin of the major proteins found in boar seminal plasma.

METHODS

Boar semen was collected with an artificial vagina from five miniature boars in routine service at the University Experiment Station. The gel was removed immediately by filtration through cheese-cloth and the spermatozoa were removed by centrifuging at 6600 g at room temperature. The seminal fluid was re-centrifuged at 10,300 g and the supernatant fluid stored at -20°C until required.

Semen was collected weekly for 3 months from Boar 1, from which the bulbo-urethral glands had been surgically removed. The seminal vesicles were then also removed and semen was collected periodically for 3 months.

Semen was collected for 3 months from Boar 2, from which the seminal vesicles had been removed. The liquid contents of the excised seminal vesicles were stored at -20°C until used.

Seminal fluid was collected for 3 months from Boar 3 which had been vasectomized.

Seminal fluid was collected for 3 months from Boar 4 from which both the bulbo-urethral and seminal vesicular glands had been removed and which had been subsequently vasectomized. This secretion is referred to as prostatic-urethral fluid.

Each biochemical analysis was based on a pooled sample consisting of at least six ejaculates per boar per treatment, except those on prostatic-urethral fluid where four ejaculates per boar constituted the sample.

Six boar epididymides obtained from the abattoir were dissected so that the vas deferens-cauda epididymidis region (VDC), defined as segments D₆ and F by Crabo (1965), was isolated. These segments were homogenized in a Waring Blender with 0.005 M-phosphate buffered saline (pH 7.4) on a 10% w/v basis.

Protein was determined at extinction 280 and/or by a modification of the Biuret method (Gornall, Bardawell & David, 1949).

Haemagglutinating activity was determined as described by Bournsnel (1967) with washed sheep erythrocytes.

Horizontal starch gel electrophoresis was run using urea and formic acid-acetic acid buffer (pH 1.7) according to the method of Aschaffenburg (1966) as modified by Dostal (1968) for studying polymorphic proteins in boar seminal vesicle fluid and seminal plasma. A similar procedure was used for alkaline electrophoresis (pH 7.5), with the exception that urea and mercaptoethanol were omitted and a buffer of 0.028 M-tris plus 0.008 M-citric acid (pH 7.5) was used in the gel and 0.05 M-lithium hydroxide plus 0.191 M-boric acid (pH 8.2)

in the electrode chambers. This procedure is a modification of the method of Matousek, Dostal & Fulka (1966).

Sephadex G-200 columns were prepared and used at room temperature as described by Hunter & Nornes (1969), with the exception that 4-ml seminal samples were analysed. Approximate molecular weights of seminal components were determined according to the technique of Leach & O'Shea (1965).

RESULTS AND DISCUSSION

Protein content

The protein content of boar semen is dependent on how effectively the animal is stimulated during ejaculation. Since ejaculation takes place over a 5-min period, the volume can be quite variable, ranging from 150 to 500 ml. The protein content of various seminal fluids used in this study is presented in Table 1. Seminal plasma contained 2.7% protein before removal of the seminal vesicles and afterwards, only 0.3% protein. This indicated that the bulk of the

TABLE 1
PROTEIN CONTENT* OF VARIOUS BOAR SEMINAL FLUIDS†

<i>Seminal fluid</i>	<i>Protein (g/100 ml)</i>
Seminal plasma (whole)	2.7
Seminal vesicle fluid	10.1
SF after removal of SV	0.3
SF after removal of BU	3.6
SF after removal of SV and BU	0.4
SF after vasectomy and removal of SV and BU	0.2

SF—seminal fluid; SV—seminal vesicles; BU—bulbo-urethral glands.

* Biuret method.

† Average of at least six ejaculates, except prostatic-urethral fluid which was average of four ejaculates.

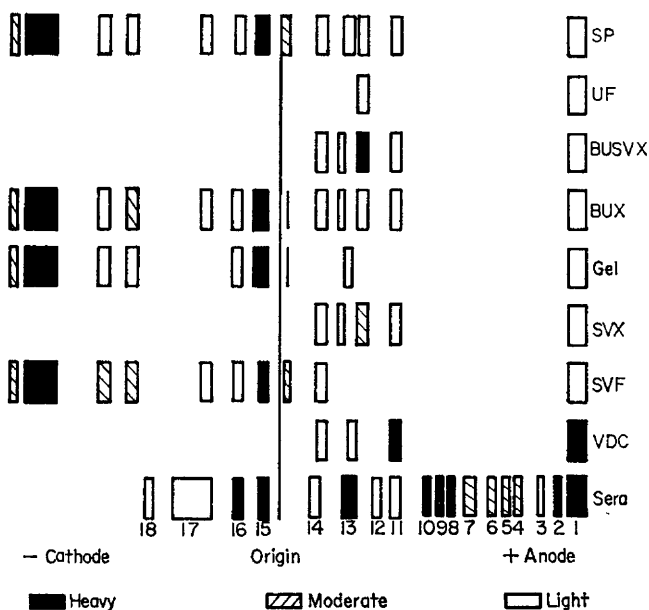
seminal proteins were of seminal vesicular origin. It appeared also that the bulbo-urethral glands contributed little if any protein, other than that in the gel, to the ejaculate. The small percentage of protein present in seminal fluid after removal of the seminal vesicles came from prostatic-urethral glands and from secretions arising from the epididymis and/or testis.

Electrophoretic behaviour of boar seminal secretions

Text-figure 1 illustrates the starch gel electrophoretic separation at pH 7.5 of various blood and seminal proteins. Swine serum separated into at least eighteen protein components. Component 1 was albumin, components 2 and 3 were post-albumins, components 4 to 6 were α -globulins, components 7 to 10 were transferrins, components 11, 12 and 14 were β -globulins, component 13 was slow α_2 -globulin and components 15 to 18 were γ -globulins. Seminal ejaculates were devoid of all serum proteins, except for traces of albumin and the slow α_2 -globulin. Seminal plasma had seven cathodic components and six anodic components at pH 7.5. Seminal vesicle fluid had the same seven cathodic

components and three of the six anodic components. Ablation of the seminal vesicles resulted in the loss of all proteins migrating to the cathode and provided proof that all of the basic proteins of seminal plasma were of seminal vesicular origin. Ablation of both the seminal vesicles and bulbo-urethral glands did not change the electrophoretic pattern from that seen after removal of only the seminal vesicles.

The electrophoretic pattern of boar gel, except for lacking one component, was identical to that of the seminal vesicle secretion. This was expected since the addition of seminal vesicle fluid to the sticky white mucin of the bulbo-urethral glands converts it into hyroscopic gelatin-like lumps (Hartree, 1962),



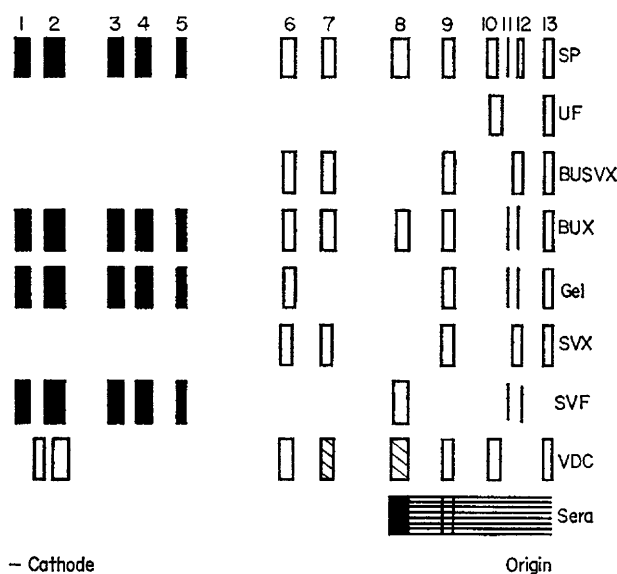
TEXT-FIG. 1. Starch gel electrophoretic patterns of various boar blood and seminal proteins under basic (pH 7.5) conditions. SP—seminal plasma; UF—prostatic-urethral fluid; BUSVX—seminal fluid after removal of BU and SV; BUX—seminal fluid after removal of bulbo-urethral glands; SVX—seminal fluid after removal of seminal vesicles; SVF—seminal vesicle fluid; VDC—vas deferens, cauda epididymidis.

which are very likely to trap soluble proteins. The sialoprotein present in bulbo-urethral mucin (Mann, 1964) would not be detected unless the mucin was first hydrolysed. Therefore, it was not possible to detect bulbo-urethral proteins electrophoretically at pH 7.5. The prostatic-urethral fluid was devoid of proteins which migrated to the cathode and contained two anodic components. Subina (1964) reported that the protein composition of boar seminal plasma was a picture of seminal vesicle proteins and those of the prostate gland. The results presented in Text-fig. 1 agreed with that general statement as eleven of the thirteen proteins detected in seminal plasma were of seminal vesicle or prostatic-urethral origin.

The VDC region was also devoid of proteins which migrated to the cathode and contained four of the six anodic components of seminal plasma. Three of

these proteins migrated electrophoretically in a similar manner to blood serum proteins and one migrated in a manner unlike any other serum protein. This protein was a seminal secretion probably arising from the VDC region or from a previous area of the tract. It was not possible to resolve this problem electrophoretically because of heavy blood contamination in extracts of epididymis and testis.

Text-figure 2 illustrates the separation of various blood and seminal proteins using starch-urea gel electrophoresis with a formic-acetic acid buffer at pH 1.7.



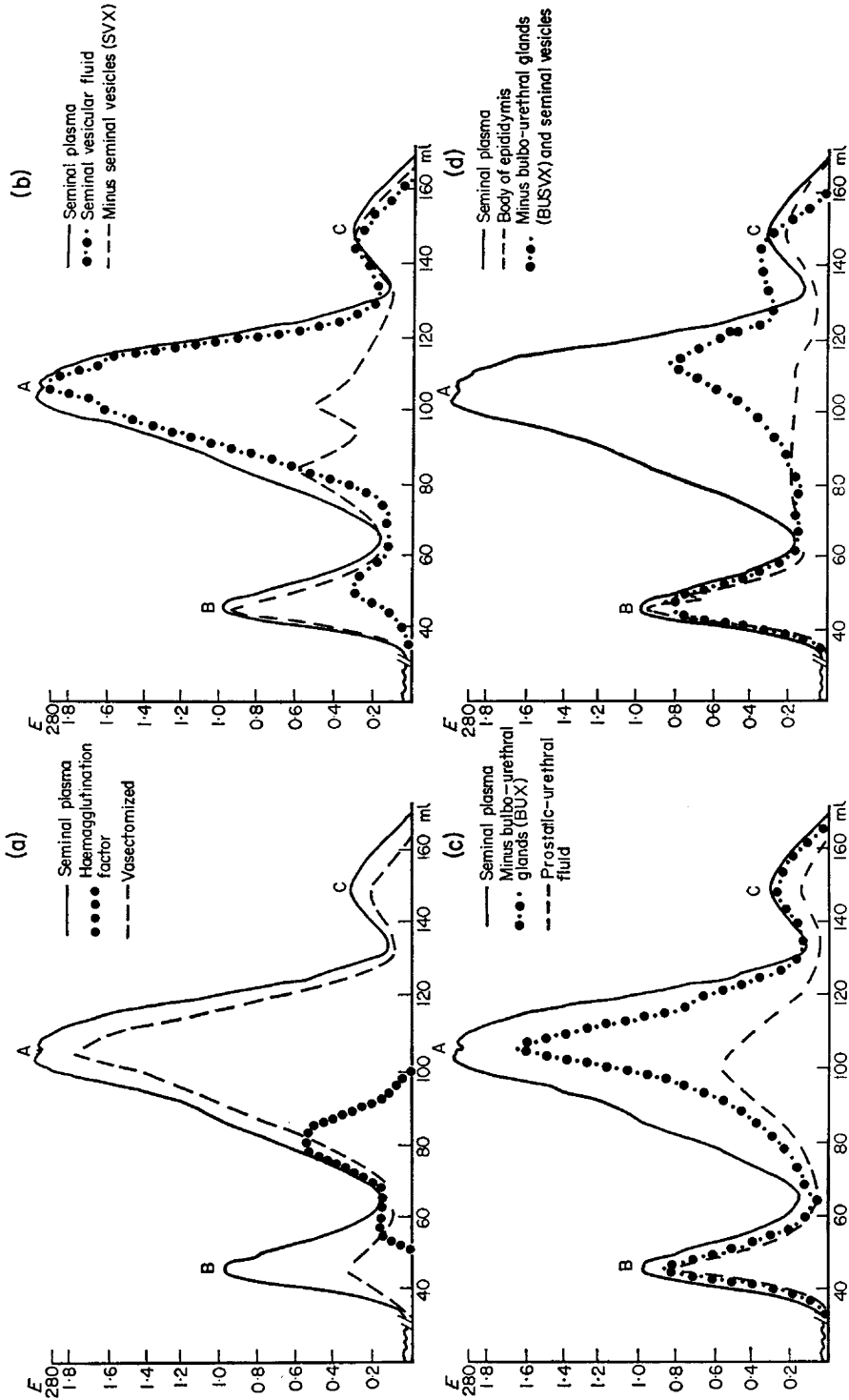
TEXT-FIG. 2. Starch gel electrophoretic patterns of various boar blood and seminal proteins under acid (pH 1.7) conditions. Abbreviations as for Text-fig. 1.

At this pH, all the proteins migrated to the cathode. Seminal plasma was resolved into thirteen components. Eight of these proteins were present in seminal vesicle fluid. Ablation of the bulbo-urethral glands did not alter the electrophoretic pattern from that of whole seminal plasma. However, ablation of both the seminal vesicles and the bulbo-urethral glands resulted in the loss of all eight of the proteins detected in seminal vesicle fluid. Component 11 of seminal vesicle fluid was probably identical with the haemagglutination factor described in papers by Bournsnel & Coombs (1966) and Bournsnel & Briggs (1969). Prostatic-urethral fluid again produced only two bands after electrophoresis in the acid buffer.

Suspensions of the VDC region contained two components (6 and 7) which were secretions from this, or previous regions, that eventually were voided in the ejaculated seminal plasma.

Behaviour of boar seminal secretions on Sephadex G-200

Text-figure 3(a) illustrates the protein elution pattern of boar seminal plasma and the haemagglutinating titre in the eluate from Sephadex G-200 columns



TEXT-FIG. 3. Diagrams of protein and haemagglutinin titre in eluates from Sephadex G-200 gel filtration of various boar seminal secretions at pH 7.5. Peaks are designated B, A, C respectively to agree with nomenclature used in literature by Boursnell.

after elution at room temperature with 0.005 M-phosphate buffered saline (pH 7.5). Haemagglutinating activity was located between Peaks A and B associated with molecules of approximately 68,000 molecular weight. This finding agreed with the report from Bournsnel *et al.* (1968). Haemagglutinating activity was absent in ejaculates from boars lacking seminal vesicles.

Text-figure 3(b) shows the protein elution patterns for seminal plasma, seminal fluid after removal of seminal vesicles, and seminal vesicle fluid. Three peaks were observed with seminal plasma corresponding to molecular weight components of approximately 155,000 (Peak B), 55,000 (Peak A) and 34,000 (Peak C). Removal of the seminal vesicular secretion resulted in a large loss only in Peak A and exposed two different groups of proteins from this region whose presence was previously masked by the bulk of seminal vesicular proteins. Therefore, Peak A was primarily of seminal vesicular origin. This was confirmed by the protein elution pattern obtained with seminal vesicular fluid (Text-fig. 3b). Peak B of seminal vesicular fluid, which was small compared to that in seminal plasma, also indicated that only a small percentage of high molecular weight proteins (155,000) were of seminal vesicular origin.

Seminal fluid obtained after ablation of both the seminal vesicles and the bulbo-urethral glands produced an elution diagram (Text-fig. 3d) in which Peaks B and C were equivalent to those found in seminal plasma. Therefore, neither the seminal vesicles nor the bulbo-urethral glands contributed appreciable protein to these two peaks. The elution diagram for prostatic-urethral fluid (Text-fig. 3c) was also very similar to that of seminal fluid for boars without bulbo-urethral and seminal vesicular glands. Hence, the bulk of the seminal proteins in the boar come from the seminal vesicles and to a much smaller extent from the prostatic-urethral glands.

The elution diagram for seminal fluid from a vasectomized boar (Text-fig. 3a) was similar to that for seminal vesicular fluid (Text-fig. 3b). Both seminal fluid from the vasectomized boar and seminal vesicular fluid showed a drastic reduction in the height of Peak B compared to A. This suggests that the majority of proteins in Peak B were of epididymal or testicular origin and the minority were of prostatic-urethral origin. This is confirmed in Text-fig. 3d, where Peak B of the eluate from the caput and corpus epididymidis was the dominant peak. Thus, the epididymis was apparently the major source of high molecular weight proteins in boar seminal plasma. The split Peak B found in each of the epididymal effluents (Text-fig. 3d) indicated that at least two groups of high molecular weight proteins are found in the epididymis. Peak C was also prominent in the epididymal secretions.

ACKNOWLEDGMENTS

This paper is Journal Article No. 7115 from the Minnesota Agricultural Experiment Station. This investigation was supported in part by USPHS Grant HD-00816.

Sincere thanks are extended to Dr Edmund F. Graham and Robert Bower, Jr, for providing access to the boar semen used in this study and to Jaromir Dostal for performing the electrophoretic analyses.

Part of this data was presented by the senior author to the University of Minnesota in partial fulfilment of the requirements for an M.S. degree.

REFERENCES

- ASCHAFFENBURG, R. (1966) Modified procedure of starch gel electrophoresis of beta-casein phenotyping. *J. Dairy Sci.* **49**, 1284.
- BOURSNELL, J. C. (1967) Boar seminal haemagglutinin. II. Combination with red cells and spermatozoa. *J. Reprod. Fert.* **13**, 297.
- BOURSNELL, J. C. & BRIGGS, P. A. (1969) Boar seminal plasma proteins. II. Electrophoretic identification of the haemagglutinin. *J. Reprod. Fert.* **16**, 457.
- BOURSNELL, J. C., BRIGGS, P. A. & COLE, D. M. (1968) Boar vesicular secretions: Further comparisons with seminal plasma proteins. *J. Reprod. Fert.* **16**, 457.
- BOURSNELL, J. C. & COOMBS, R. R. A. (1966) A haemagglutinating factor in boar seminal plasma. *J. Reprod. Fert.* **11**, 139.
- BOURSNELL, J. C., JOHNSON, P. & ZAMORA, J. (1962) An electrophoretic and ultracentrifugal study of boar seminal plasma. *Biochim. biophys. Acta*, **63**, 374.
- CRABO, B. (1965) Studies on the composition of epididymal content in bulls and boars. *Acta vet. scand.* **6**, Suppl. 5.
- DOSTAL, J. (1968) A study on polymorphic proteins of seminal vesicle fluid and seminal plasma in boars. *Immunogenet. Lett.* **5**, 117.
- GORNALL, A. G., BARDAWELL, C. J. & DAVID, M. M. (1949) Determination of serum proteins by means of the biuret reaction. *J. biol. Chem.* **177**, 751.
- HARTREE, E. F. (1962) Sialic acid in bulbo-urethral glands of the boar. *Nature, Lond.* **196**, 483.
- HUNTER, A. G. & NORNES, H. O. (1969) Characterization and isolation of a sperm-coating antigen from rabbit seminal plasma with capacity to block fertilization. *J. Reprod. Fert.* **20**, 419.
- LEACH, A. A. & O'SHEA, P. C. (1965) The determination of protein molecular weights of up to 225,000 by gel-filtration on a single column of Sephadex G-200 at 25° and 40°. *J. Chromat.* **17**, 245.
- MANN, T. (1964) *The biochemistry of semen and the male reproductive tract*. John Wiley, New York.
- MATOUSEK, J., DOSTAL, J. & FULKA, J. (1966) *Antigenicity and polymorphism of the seminal vesicle fluid in boars*. Polymorphismes biochimiques des animaux. Xth Congrès européen sur les groupes sanguins et le polymorphisme biochimique des animaux, Paris, p. 523.
- NELSON, M. & BOURSNELL, J. C. (1966) Studies on boar seminal plasma proteins. 4. Isolation of factors with haemagglutinating and protein-precipitating activity. *Biochim. biophys. Acta*, **117**, 144.
- SUBINA, G. N. (1964) Nasledstvennost i izmencivost selskochozjastvennykh zivotnykh. pp. 121-131. Leningrad: Kolos.