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Effect of bovine serum albumin on motility and fecundity of turkey spermatozoa before and after storage

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Summary. Motility characteristics of turkey spermatozoa before and after storage for 24 h at 7°C in diluent with and without bovine serum albumin (BSA; 1% final concentration) were measured by computer-assisted semen analysis. BSA significantly increased the percentage of motile spermatozoa and sperm velocity, linearity, lateral head displacement and beat frequency in each treatment, but BSA in fresh or stored semen in diluent did not augment hen fertility over 15 weeks of egg production. Fatty-acid-free BSA, globulin-free BSA and Fraction V BSA all significantly increased each sperm motility characteristic compared with semen in diluent alone. The lack of correlation between sperm motility and fecundity emphasizes the need to develop procedures for semen evaluation that accurately predict the fertilizing capacity of an aliquot of semen.

Keywords: artificial insemination; sperm motility; serum albumin; fertility; semen; turkey

Introduction

Work by Lake & Ravie (1987) indicated that chicken semen subjected to a high dilution rate (46:1) with seminal plasma obtained from the ductus deferens retained its fecundity. Remarkably, these investigators obtained excellent fertility, inseminating only $5{\cdot}45 \times 10^6$ sperm immediately after dilution. Lake & Ravie (1987) attributed their success to an instant sperm motility stimulus afforded by an unknown factor(s) associated with the suspension of spermatozoa in the seminal plasma diluent.

Investigations using semen from various mammalian species have indicated that bovine serum albumin (BSA) stimulates sperm motility by an unknown mechanism (Harrison *et al.*, 1982; Klem *et al.*, 1986). In this study, we examined the effects of BSA on turkey sperm motility and fecundity. Preliminary studies in our laboratory indicated that BSA boosts motility of turkey spermatozoa instantly. It was our objective to verify these initial observations and then to determine whether such a boost in motility enhances hen fertility before and after storage of semen for 24 h at 7°C.

A major problem in estimating sperm motility is that the procedures most commonly used are subjective. Generally, an observer views some dilution of semen at ambient temperature and, using some predetermined criteria, estimates the percentage of motile spermatozoa. Problems arise when a second individual views the same sample and either uses a different set of criteria to score motility or, using the same set of criteria, assigns a different score. Problems due to subjectivity in semen evaluation techniques have been addressed elsewhere (Bakst & Cecil, 1989). For such a subjective procedure to be meaningful, all aspects of each semen evaluation procedure must be standardized and understood by those performing the procedure within and between laboratories.

One method developed to measure sperm motility objectively involves the use of video and computer technology. Such systems, which are available commercially, analyse individual spermatozoa on sequential video frames and rapidly derive the percentage motility, sperm velocity,

linearity (an index of path straightness), lateral head displacement and beat frequency (the last two describe amplitude and frequency of sperm head/tail beat).

Little has been resolved regarding the relationship between turkey sperm motility and fecundity and our preliminary observations showed that BSA augments turkey sperm motility. We investigated whether BSA-enhanced sperm motility results in higher fertility. We also investigated the effects of fatty-acid-free and globulin-free BSA on turkey sperm motility.

Materials and Methods

Animals

One-day-old commercial Large White breeder turkey tom and hen poults were purchased from a primary breeder and maintained under standard husbandry conditions during their brooding and growth periods. Toms and hens were photostimulated (03:00–17:00 h light) at 26 and 30 weeks of age, respectively. Semen was first manually collected from toms at 28 weeks of age and, thereafter, at least once a week using two cloacal strokes (Bakst & Cecil, 1983). All semen collections consisted of pooled ejaculates from 15–20 toms. Within 30 min of collection, the pooled semen was diluted 1:1 with turkey semen diluent (SemAid; Poultry Health Laboratories, Davis, CA, USA).

Visual and computer-assisted evaluation of sperm motility

Visual estimates of spermatozoa were made by one observer within 1 h of semen dilution. Progressive motility was estimated (scale 0–100) from a 10 µl drop of the 1:1 diluted semen placed on a slide with a coverslip at ambient temperature and examined with a phase-contrast microscope (× 400).

The CellSoft (Cryo Resources Ltd, New York, NY, USA) system was used to assess objectively certain characteristics of turkey sperm motility: velocity, linearity (an index of path straightness; a value of 10 represents a straight path, and decreasing values represent further degrees of nonlinearity), mean lateral head displacement (an amplitude measurement providing an estimation of the perpendicular distance the sperm head deviates from the computer-generated sperm path) and beat frequency (a sperm tail-beat frequency measurement based on the number of times the sperm head crosses the computer-generated sperm path). The CellSoft system derives this information from video images (either real time or from play-back video recordings), which are captured sequentially (30 frames/s) and digitized by the computer. The digitized video frames are then analysed as directed by the software parameters defined in Table 1.

Table 1. Set-up parameters for the quantification of turkey sperm motility characteristics using the CellSoft system

Camera frame rate (Hz)	30
Number of consecutive frames to analyse	15
Minimum number of consecutive frames to determine:	
motility	4
velocity	15
lateral head displacement	7
beat frequency	7
Pixel scale (µm/pixel)	0·654
Threshold velocity (µm/s)	10
Threshold grey	42
Maximum/minimum velocity (µm/s)	300/10
Cell size range (pixels)	25–90

CellSoft analyses were conducted at ambient temperature using semen diluted with the appropriate treatment diluent to about 25 × 10^6 spermatozoa/ml. (Preliminary work indicated that 25 × 10^6 spermatozoa is the optimal concentration that can be used with the CellSoft system.) To obtain this concentration, 6 µl of semen diluted 1:1 with the appropriate treatment diluent was added to 1 ml of the same diluent, dispersed gently and evaluated within 30 min. The semen (6 µl) was placed on a Makler Counting Chamber (CryoResources Ltd, New York, USA),

and allowed to settle for 1 min before evaluation. Twelve microscopic fields were defined relative to the grid surface on the counting chamber. Each field with spermatozoa (up to a total of 300 spermatozoa) was sequentially recorded for future analysis.

To determine the effects of BSA on sperm motility before and after storage for 24 h, another 5 ml of pooled semen was obtained and mixed thoroughly before splitting into two aliquots. An equal volume of diluent or diluent plus BSA was added to the semen and mixed thoroughly and the fresh semen was evaluated. At this point, the diluent aliquot was further divided into two parts and prepared for storage as described in the next section. After storage for 24 h at 7°C, each part was diluted again, 1:1 with diluent or diluent plus BSA, and the motility characteristics were analysed by the CellSoft system.

Preparation of semen for insemination

To determine whether the presence of BSA (A4378, Sigma Chemical Co., St Louis, MO, USA) in the semen diluent enhanced hen fertility with fresh semen or semen stored for 24 h, 5 ml of pooled semen was mixed thoroughly before splitting into two aliquots. An equal volume of diluent or diluent plus BSA (all BSA aliquots in this experiment had a final concentration of 1%) was added to the semen and mixed thoroughly. Each aliquot was divided again, one part used for insemination within 90 min of collection, and the other part stored for 24 h and then used for insemination. For storage, semen aliquots were kept in 10-ml Erlenmeyer flasks covered loosely with foil. These flasks were placed in beakers containing enough water to reach the upper level of the semen in the flasks and then the beakers were placed on an orbital shaker (150 r.p.m.) in a refrigerator at 7°C (Sexton, 1987).

Four treatment groups consisting of 12 hens/group were established: fresh semen in diluent; stored semen in diluent; fresh semen in diluent plus BSA; stored semen in diluent plus BSA. Hens were inseminated initially on days 14 and 15 after the onset of photostimulation and once a week thereafter for the next 14 weeks with 100×10^6 viable spermatozoa. The percentage of live (viable) spermatozoa was determined using the ethidium bromide exclusion procedure (Bilgili & Renden, 1984). The insemination dose was adjusted accordingly to include a constant number of viable spermatozoa with each insemination. Eggs were collected three times a day, set once a week and candled at 7–10 days of incubation.

Effects of different BSA preparations on sperm motility

The effects of four BSA preparations (Sigma, St Louis, MO, USA) were evaluated, including (i) BSA (Sigma A4378), also used in the above experiment, containing fatty acids and 1–3% globulins; (ii) fatty-acid-free (<0·005%) BSA (Sigma A7511; prepared from A4378); (iii) globulin-free BSA (Sigma A7638); and (iv) Fraction V BSA (Sigma A6793), an inexpensive BSA preparation. Stock solutions of 2% BSA in diluent were prepared and refrigerated.

For the CellSoft analyses, 2 ml of pooled semen was mixed thoroughly and 200 µl was placed in five cryovials suspended in an ice–water bath. Four semen aliquots were diluted 1:1 with a BSA preparation and one with diluent. The samples were then placed on an orbital shaker (150 r.p.m.) in a refrigerator at 7°C for a minimum of 30 min before CellSoft evaluations.

Statistical analysis

Analysis of variance was conducted to determine significant differences among treatments. If the F value was significant ($P \leq 0{\cdot}05$), treatment means were separated using a least-squares difference ($P = 0{\cdot}05$).

Results

Effect of BSA on sperm motility

Analysis of variance indicated that highly significant differences ($P < 0{\cdot}01$) were associated with each diluent treatment within each sperm motility characteristic except for lateral head displacement ($P = 0{\cdot}36$) (Table 2). With BSA in the diluent, fresh semen exhibited slightly more than twice the percentage of motile sperm and 2–3 times the score for linearity and beat frequency, but no change in velocity, compared with fresh semen and diluent. Only linearity showed any significant change in sperm motility after 24 h storage in diluent without BSA. Sperm stored in BSA had increased motility, velocity and linearity. The same magnitude of increase could also be accomplished by addition of BSA to diluted semen after storage for 24 h. Although the beat frequencies of the two stored semen samples containing BSA were similar to the beat frequency of

those stored in diluent without BSA after storage for 24 h, sperm velocities of the stored samples containing BSA were significantly greater than those without BSA.

Effect of BSA on sperm fecundity before and after storage

Analysis of variance indicated no variance in hen group fertility ($P = 0{\cdot}18$) due to type of diluent (with or without BSA), but there was a significant effect ($P = 0{\cdot}002$) due to storage of the semen (fresh or stored for 24 h). There was no diluent × storage interaction ($P = 0{\cdot}29$).

Although the percentage fertility of the hen group inseminated with diluent alone remained unchanged during the egg production period examined, there were highly significant period effects ($P = 0{\cdot}0001$), period × diluent ($P = 0{\cdot}0001$) and period × storage ($P = 0{\cdot}001$) interactions. Not only was no benefit derived from the use of BSA in diluent with either the fresh or stored semen, but also the presence of BSA had a significant depressing effect on hen fertility, particularly evident in the last two egg-production periods (Table 3).

Effect of different BSA preparations on sperm motility

All BSA preparations significantly increased each motility characteristic examined when compared with diluent without BSA (Table 4). Compared with the globulin-free BSA, fatty-acid-free BSA resulted in a significant increase in the percentage visual motility and the percentage motility, velocity and linearity. Although visual motility of the Fraction V BSA was not different from the fatty acid-free BSA, the CellSoft percentage motility, velocity and linearity of the Fraction V BSA were significantly lower than those of the fatty-acid-free BSA.

Discussion

While the presence of BSA in fresh and stored turkey semen samples significantly increased several sperm motility characteristics, overall hen fertility was not affected by BSA. This does not necessarily contradict the results of Lake & Ravie (1987) (see Introduction) since they inseminated few sperm and their diluent was homologous ductus deferens seminal plasma, which not only may affect sperm motility but also may affect other sperm functions relative to successful oviductal sperm transport and selection. The effect of seminal plasma on turkey sperm motility patterns remains to be described; but the percentage of motile sperm, and sperm velocity and linearity were significantly higher in all treatments containing BSA than in those using diluent alone. This effect was further enhanced after semen storage for 24 h.

It is speculated that the enhanced motility characteristics of stored sperm may reflect the presence of a subpopulation of sperm capable of surviving storage for 24 h under the described conditions (see Materials and Methods). Of particular interest is the response of sperm to the BSA when added at the end of the storage period. The sperm motility characteristics were similar to those of sperm stored in BSA for 24 h, suggesting that a subpopulation of sperm capable of responding to a sperm-motility stimulus exists in the pooled semen sample before and after storage for 24 h. These sperm may represent that small population selected by the oviduct and stored by the oviductal sperm-storage tubules (Brillard & Bakst, 1990).

This select population of sperm continued to respond to BSA after storage for 24 h, but the specific increases in sperm velocity and linearity were associated with a decline in hen fertility over the 15 weeks of egg production. In contrast, other semen evaluation procedures, such as sperm morphology, visual estimation of motility, and live/dead procedures fail to indicate differences in semen quality before and after storage (Sexton, 1988; Wishart & Steele, 1990).

It is not evident how the BSA-induced increase in sperm velocity and the improved linearity of the sperm path are detrimental to fertility. Possibly changes in these motility characteristics affect sperm interaction with oviductal luminal secretions and/or cilia and alter the efficacy of sperm

Table 2. Changes in motility characteristics of turkey spermatozoa recorded by the CellSoft system due to diluent with or without bovine serum albumin (BSA), before (fresh) and after storage for 24 h, compared with fresh semen in diluent alone

Treatments[1]	CellSoft system readings				
	Motility	Velocity	Linearity	Mean lateral head displacement	Beat frequency
Diluent No BSA					
Fresh	$1{\cdot}00^{c}$ (10%)[3]	$1{\cdot}00^{b}$ (37·3 μm/s)	$1{\cdot}00^{c}$ (1·8)	$1{\cdot}00^{a}$ (1·1 μm)	$1{\cdot}00^{b}$ (6·3 Hz)
Stored + Diluent[2]	$1{\cdot}58^{bc}$	$0{\cdot}94^{b}$	$2{\cdot}34^{b}$	$1{\cdot}18^{a}$	$2{\cdot}92^{a}$
Stored + BSA[2]	$4{\cdot}96^{a}$	$1{\cdot}58^{a}$	$4{\cdot}05^{a}$	$1{\cdot}68^{a}$	$2{\cdot}88^{a}$
Diluent + BSA					
Fresh	$2{\cdot}38^{b}$	$1{\cdot}12^{b}$	$2{\cdot}75^{b}$	$1{\cdot}61^{a}$	$2{\cdot}69^{a}$
Stored	$4{\cdot}81^{a}$	$1{\cdot}63^{a}$	$3{\cdot}99^{a}$	$1{\cdot}55^{a}$	$3{\cdot}08^{a}$
Least-squares difference	0·88	0·38	0·77	0·87	0·80

Means in columns with different superscripts are significantly different ($P < 0{\cdot}05$).
[1]There were six replicates per treatment.
[2]Semen was stored in diluent for 24 h and then further diluted 1:1 with diluent (stored + diluent) or with BSA in diluent (stored + BSA).
[3]Values in parentheses are means of replicates describing sperm motility characteristics for 'Diluent - No BSA' treatment.

Table 3. Effect of diluent with and without bovine serum albumin (BSA) on fertility of turkey hens before (fresh) and after storage of semen for 24 h at 7°C

Treatments	Percentage fertility in egg production periods (weeks)					
	1–3	4–6	7–9	10–12	13–15	1–15
Diluent						
Fresh	$90^{a-d}\pm3$	$90^{a-d}\pm3$	$93^{a-c}\pm3$	$90^{a-d}\pm2$	$93^{a-c}\pm3$	91 ± 2 (835)[1]
Stored	$89^{a-e}\pm2$	$90^{a-d}\pm2$	$74^{ef}\pm6$	$60^{hi}\pm9$	$65^{f-i}\pm6$	77 ± 4 (843)
Diluent + BSA						
Fresh	$95^{a}\pm2$	$89^{a-d}\pm4$	$85^{c-e}\pm5$	$74^{ef}\pm12$	$72^{f-h}\pm6$	88 ± 2 (661)
Stored	$94^{ab}\pm2$	$86^{b-e}\pm4$	$75^{d-f}\pm9$	$52^{ij}\pm10$	$47^{j}\pm7$	75 ± 6 (792)

[1]Numbers in parentheses are the numbers of eggs set in the incubators over the 15 weeks of egg production.
[a-j]Based on least-squares difference of arcsine-transformed data, means (±s.e.m.) with different superscripts are significantly different ($P < 0{\cdot}05$).

transport to acceptance by the sperm-storage tubules. Accelerated motility may propel the sperm past the sperm-storage tubules to the more anterior regions of the oviduct. An alternative explanation for its detrimental effect on fertility is that BSA may be coating the sperm, thereby partially masking surface proteins that appear to be involved in oviductal sperm selection and transport to the sperm-storage tubules (Wishart & Steele, 1990).

There is no question that BSA increased several measurable characteristics describing turkey sperm motility. Similar observations have been reported with sperm of other species. The presence of BSA in media used to support boar, ram, rabbit and stallion semen augmented sperm motility (Harrison *et al.*, 1978). The last authors conducted an extensive investigation attempting to identify the specific components of BSA that stimulated sperm motility. Defatting the BSA and mild denaturation of the BSA did not diminish its effect on sperm motility; BSA did not show chelating properties that could affect sperm motility (Harrison *et al.*, 1978, 1982). In contrast, Dixon &

Table 4. Changes in motility characteristics of turkey spermatozoa due to treatments with four types of bovine serum albumin (BSA) compared with diluent alone estimated visually and by the CellSoft system

	Visual	CellSoft system readings				
BSA treatment	Motility	Motility	Velocity	Linearity	Mean lateral head displacement	Beat frequency
Diluent – No BSA	1·00^{c} (19) (40%)[1]	1·00^{d} (21) (7%)	1·00^{d} (31 μm/s)	1·00^{d} (2·3)	1·00^{b} (1·4 μm)	1·00^{b} (14·1 Hz)
Fatty-acid-free BSA	1·69^{a} (15)	5·42^{a} (18)	1·45^{a}	2·70^{a}	1·21^{a}	1·19^{a}
Fraction V	1·58^{a} (19)	5·18^{ab} (22)	1·36^{ab}	2·35^{b}	1·18^{a}	1·13^{a}
Globulin-free	1·53^{a} (15)	3·32^{c} (18)	1·24^{bc}	1·87^{c}	1·23^{a}	1·11^{a}
Least-squares difference	1·27^{b} (19)	3·89^{bc} (21)	1·21^{c}	1·83^{c}	1·17^{a}	1·10^{ab}
	0·22	1·36	0·13	0·26	0·16	0·11

Means in columns with different superscripts are significantly different ($P < 0·05$).
Numbers in parentheses are the number of observations per treatment for the visual estimate of motility and for the CellSoft observations.
[1]Values in parentheses in this row are means of replicates describing sperm motility characteristics for 'Diluent – No BSA' treatment.

Kreider (1981) noted that stallion sperm motility only increased in the presence of BSA with free fatty acids and they suggested that the fatty acids served as metabolic substrates.

Although nearly all the BSA preparations, when compared with diluent alone, significantly increased each of the turkey sperm motility characteristics, fatty acid-free BSA was the most potent effector and globulin-free BSA was the least potent effector of turkey sperm motility. While a metabolic response to the free fatty acids in BSA resulting in an increase in turkey sperm motility is unlikely, the precise mechanism that results in the augmentation of turkey sperm motility remains speculative. Our observations support the statement by Harrison *et al.* (1982) that attributes BSA with the ability to stimulate the motility of sperm from several different species, which now includes turkey.

A number of observations indicate that BSA is adsorbed to the sperm plasma membrane. Blank *et al.* (1976) stated that BSA coats about half the surface area of rabbit sperm in conditions that approximate those necessary for *in vitro* capacitation. Harrison *et al.* (1978, 1982) and Dott *et al.*, (1979) alluded to the possibility that BSA may coat sperm since they noted that fewer sperm adhered to the slide when diluted with BSA-containing media. Although we did not specifically examine the frequency of sperm sticking to a slide, the observed increase in certain motility characteristics would suggest that turkey sperm may be coated with the BSA. Such a coating may render the sperm less subject to physical obstacles that could hinder motility. It has been suggested by Tchacarof & Mollova (1980) that a BSA–sperm surface interaction rendered ram sperm more resistant to the otherwise deleterious effect of a high rate of semen dilution. Bovine serum albumin appears to have a beneficial effect on turkey sperm subject to a semen dilution rate high enough to get a concentration of 25×10^6 sperm/ml (see Materials and Methods). The percentage of motile sperm in diluent without BSA (Table 2) indicates that these sperm were subject to the classic dilution effect (see Bakst, 1990).

Although sperm velocity and percentage of motile sperm was enhanced by the addition of BSA to the diluent there was no increase in percentage of fertile eggs from hens inseminated with semen diluted with BSA. This lack of a correlation between hen fertility and sperm motility has been observed previously (Cherms, 1968; Sexton & Giesen, 1983) and further underscores the need to develop semen evaluation procedures which can more accurately predict the fertilizing capacity of an aliquot of fresh or stored turkey semen.

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References

Bakst, M.R. (1990) Preservation of avian cells. In *Poultry Breeding and Genetics*, pp. 91–108. Ed. R. D. Crawford. Elsevier Science Publishers B. V., Amsterdam.

Bakst, M.R. & Cecil, H.C. (1983) Gross appearance of turkey cloacae before and after single or multiple manual semen collections. *Poultry Sci.* **62,** 683–689.

Bakst, M.R. & Cecil, H.C. (1989) Evaluation of turkey semen. *Proc. Int. Symp. Turkey Reprod.*, June 12–14, 1989, Department of Poultry Science, North Carolina State University, Raleigh, NC, USA.

Blank, M., Soo, L. & Butten, J.S. (1976) Adsorption of albumin on rabbit sperm membranes. *J. Membrane Biol.* **29,** 401–409.

Bilgili, S.F. & Renden, J.A. (1984) Fluorometric determination of avian spermatozoal viability and concentration. *Poultry Sci.* **63,** 2275–2277.

Brillard, J.P. & Bakst, M.R. (1990) Quantification of spermatozoa in the sperm-storage tubules of turkey hens and the relation to sperm numbers in the perivitelline layer of eggs. *Biol. Reprod.* **43,** 271–275.

Cherms, F.L. (1968) Variations in semen quality and the relationship of semen quality to fertility in turkeys. *Poultry Sci.* **47,** 746–754.

Dixon, K.E. & Kreider, J.L. (1981) Effect of calcium and fatty acids on the isolation of stallion spermatozoa in BSA. *Theriogenology* **15,** 335–341.

Dott, H.M., Harrison, R.A.P. & Foster, G.C.A. (1979) The maintenance of motility and the surface properties of epididymal spermatozoa from bull, rabbit and ram in homologous seminal and epididymal plasma. *J. Reprod. Fert.* **55,** 113–124.

Harrison, R.A.P., Dott, H.M. & Foster, G.C. (1978) Effect of ionic strength, serum albumin and other macromolecules on the maintenance of motility and the surface of mammalian spermatozoa in a simple medium. *J. Reprod. Fert.* **52,** 65–73.

Harrison, R.A.P., Dott, H.M. & Foster, G.C. (1982) Bovine serum albumin, sperm motility and the 'Dilution Effect'. *J. exp. Zool.* **222,** 81–88.

Klem, M.E., Kreider, J.L., Pruitt, J.B. & Potter, G.D. (1986) Motility and fertility of equine spermatozoa extended in bovine serum albumin and sucrose. *Theriogenology* **26,** 569–576.

Lake, P.E. & Ravie, O. (1987) Effect on fertility of low numbers of fowl spermatozoa inseminated in aqueous diluent or semen components of the fowl and turkey. *Br. Poultry Sci.* **28,** 75–80.

Sexton, T.J. (1987) Effect of semen treatments and age of tom on fertility of unstored semen and semen held 18 hours. *Poultry Sci.* **66,** 1721–1726.

Sexton, T.J. (1988) Comparison of commercial diluents for holding turkey semen 24 hours at 5°C. *Poultry Sci.* **67,** 131–134.

Sexton, T.J. & Giesen, A.F. (1983) Beltsville Poultry Semen Extender. 8. Factors affecting semen held six hours at 15°C. *Poultry Sci.* **62,** 1063–1068.

Tchacarof, E.L. & Mollova, M.V. (1980) Influence of bovine serum albumin (BSA) on the motility of spermatozoa upon high-degree dilutions applied in photokinesigraphy. *C.R. Acad. Bulgrade Sci.* **33,** 655–658.

Wishart, G.J. & Steele, M.G. (1990) The influence of sperm surface characteristics on sperm function in the female reproductive tract. In *Proceedings, 'Control of Fertility in Domestic Birds'*, Tours, France, 2–4 July 1990, pp. 101–112. Ed. J. P. Brillard (Les Colloques de l'INRA, No. 54).

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