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Original article

Effect of indole-3-acetic acid (plant auxin) on the preservation at 15 °C of boar semen for artificial insemination

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Summary — In order to extend the duration of boar sperm survival at 15 °C for artificial insemination, we tested the effect of indole-3-acetic acid (IAA), which appeared to be the main sperm protective substance present in the *Coco nucifera* endosperm (coconut water). Two IAA concentrations (10 and 100 ng/mL) in Beltsville extender (BTS) were studied for their in vitro effects. The motility, the percentage of motile spermatozoa and the acrosome morphology of sperm were recorded each day over 13 days of storage at 15 °C, after 5 min and 3 h of incubation at 39 °C. The IAA effect on sperm preservation was also studied in vivo at a concentration of 10 ng/mL in BTS by inseminating groups of females twice at 24 h intervals either at D0 (day of sperm collection) and D1 (D0/1) or at D5 and D6 (D5/6). At D0/1, the two groups of females (control and IAA) were inseminated with a total of 6.3 x 10^9 spermatozoa (3.15 x 10^9 at D0 and the same dose at D1) whereas at D5/6, one IAA group was inseminated with a total of 6.3 x 10^9 spermatozoa and another one with 12.6 x 10^9 spermatozoa. The animals in the D5/6 control group were inseminated each with a total of 12.6 x 10^9 spermatozoa. For each group of females (n = 106–140), fertility rate (% farrowing) and prolificacy rate (litter size) were recorded. No effect of IAA in vitro on the motility rate and on the percentage of motile spermatozoa was observed over a 13 day storage. However, IAA (10 ng/mL) had a significant positive effect on the percentage of living spermatozoa with intact acrosomes after 13 days (66 vs 54%, $P < 0.05$). The fertility and prolificacy rates after 5–6 days of sperm preservation in BTS extender alone did not differ significantly between D5/6 and D0/1 but the total number of inseminated spermatozoa was 12.6 x 10^9 at D5/6 instead of 6.3 x 10^9 at D0/1. When the spermatozoa were stored in the presence of 10 ng/mL IAA for 5–6 days at 15 °C, the fertility and prolificacy of the females inseminated with only 6.3 x 10^9 spermatozoa were identical to those of the females inseminated with an equal number of spermatozoa at D0/1 in the presence or absence of IAA.

pig / sperm / 3-indole-acetic acid / fertility / prolificacy / artificial insemination

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Résumé — Effet de l'auxine végétale, l'acide 3-indole-acétique, sur la conservation du sperme de verrat pour l'insémination artificielle. *Afin de prolonger la durée de conservation du sperme de verrat à 15 °C pour l'insémination artificielle, nous avons étudié l'effet de l'acide 3-indole acétique (IAA) que nous avons identifié comme la principale substance protectrice du sperme présente dans l'endosperme de* Coco nucifera *(eau de coco). Deux concentrations d'IAA (10 et 100 ng/mL) dans le dilueur de Beltsville (BTS) ont été étudiées in vitro. La mobilité et le pourcentage de spermatozoïdes mobiles ainsi que l'intégrité de leurs acrosomes ont été étudiés sur une période de 13 jours de conservation à 15 °C après des incubations de 5 minutes et 3 heures à 39 °C. L'effet de l'IAA a aussi été étudié in vivo à la concentration de 10 ng/mL de BTS en inséminant des groupes de femelles deux fois soit, le jour de la collecte et le lendemain (j0/1) soit, aux cinquième et sixième jours après la collecte (j5/6). À j0/1, les femelles des deux groupes (témoin et IAA) ont été inséminées avec un total de 6,3 x 10^9 spermatozoïdes (3,15 x 10^9 à j0 et j1) tandis qu'à j5/6 les femelles du groupe IAA ont été inséminées avec 6,3 x 10^9 spermatozoïdes (3,15 x 10^9 à j5 et j6) et les femelles des deux autres groupes (témoin et IAA) par 12,6 x 10^9 spermatozoïdes (6,3 x 10^9 à j5 et à j6). La fertilité et la prolificité des animaux de chaque groupe (n = 106–140) ont été enregistrées. Aucun effet de l'IAA in vitro n'a été observé sur la mobilité massale et le pourcentage de spermatozoïdes mobiles sur une période de 13 jours. Cependant, l'IAA (10 ng/mL) a un effet favorable significatif sur le pourcentage de spermatozoïdes vivants dont l'acrosome est intact après 13 jours (66 % versus 54 % ; $p < 0,05$). Aucune baisse de la fertilité et de la prolificité n'est observée chez les truies inséminées avec 12,6 x 10^9 spermatozoïdes conservés pendant 5–6 jours par rapport à celles inséminées par 6,3 x 10^9 spermatozoïdes conservés 0–1 jour. La fertilité et la prolificité des truies inséminées par seulement 6,3 x 10^9 spermatozoïdes conservés pendant 5-6 jours en présence d'IAA (10 ng/mL) à 15 °C sont les mêmes que celles des animaux inséminés par le même nombre de spermatozoïdes conservés seulement pendant 0–1 jour avec ou sans IAA.*

porc / spermatozoïde / acide 3-indole-acétique / fertilité / prolificité / insémination artificielle

INTRODUCTION

Artificial insemination (AI) in the porcine species is usually performed with boar semen stored at 15 °C for up to a maximum of 3 days, after which time a significant decrease in fertilizing ability is observed (Paquignon, 1984). For an efficient organization of inseminations and optimal use of semen doses in breeding, it is essential that sperm can be stored over long periods of time with no or minimal loss in fertilizing ability (Paquignon et al, 1982a; Rillo et al, 1991). The best current extender for porcine AI is Beltsville thawing solution (BTS) (Paquignon et al, 1987; Blichfeld et al, 1988; Reed and Curnock, 1991) which permits economically acceptable boar sperm preservation for 2–3 days after collection (Paquignon et al, 1988). Taking into consideration work organization in pig farms, it would be of great interest to conserve fertile sperm over a 5–6 day period.

Preliminary studies have shown that endosperm from *Coco nucifera* (coconut water) can be used for the preservation of sperm from bucks (Toniolli, 1989), boars (Toniolli, 1990, 1991) and rams (Freitas and Ferreira-Nunès, 1994). Fractionation of coconut water has permitted isolation of a component, named JYP, which appeared to be, at least in part, responsible for the favourable effect of coconut water (Ferreira-Nunès, Combarnous and Leclercq, unpublished data). Later, indole-3-acetic acid (IAA) was identified as the main active molecule in JYP (Combarnous and Ferreira-Nunès, 1995).

In the present paper, we report the effect of the addition of IAA to BTS extender in the survival of boar sperm at 15 °C over a 0–13 day period of time. These effects were evaluated in vitro on sperm motility parameters and acrosome morphology integrity and in vivo on fertility and prolificacy rates in

sows inseminated after 0–1 or 5–6 days of sperm conservation.

MATERIALS AND METHODS

Forty-nine boars (Landrace and Large White fullbred and crossbred) in regular service (one ejaculate per week on average) were used in the present study. In all ejaculates, sperm motility rates were ≥ 3 and percentages of motile spermatozoa were ≥ 75%.

Immediately after collection, each ejaculate was split into two (in vivo assays) or three (in vitro assays) fractions diluted at a concentration of 35 x 10^6 spermatozoa per mL in BTS extender alone or in BTS containing 10 ng/mL IAA (5.7 x 10^{-9} M) in in vivo and in vitro assays or 100 ng/mL IAA (5.7 x 10^{-8} M) in in vitro assays. The BTS extender was purchased in dry form from Cobiporc (Saint-Gilles, France) and prepared as recommended by the manufacturer.

In in vitro studies, 3 mL fractions were incubated in 5 mL glass tubes for time periods of 0 to 13 days at 15 °C. Each day, except D8, D10 and D12, one tube from each group was removed and incubated for 3 h at 39 °C in a water bath. Motility parameters were evaluated microscopically at x 128 magnification after 5 min and 3 h at 39 °C as described by Bork et al (1988). The observations were made at a concentration of 35 x 10^6 spermatozoa per mL in blind tests on at least three fields. The integrity of spermatozoa acrosome morphology was assessed on 300 to 400 spermatozoa at D13 using Congo red and Giemsa stain (Kovacs and Foote, 1992).

For the in vivo study, a total of 634 crossbred sows were used among which only 54 (8.5%) were nulliparous and evenly distributed among the five experimental groups randomly distributed among 112 farms. Forty-nine ejaculates from 37 boars were used. The experiment was carried out between December 1992 and December 1994 over all seasons. Four technicians were in charge of artificial inseminations and the collection of farrowing data. Inseminations were performed twice at 24 h intervals at days 0 and 1 (D0/1) or at days 5 and 6 (D5/6) after sperm collection. The females in the D0/1 groups (control and IAA) were inseminated with 2 x 90 mL diluted sperm (total of 6.3 x 10^9 spermatozoa). The females in two D5/6 groups (control and IAA) were inseminated with 2 x 180 mL diluted sperm (total of 12.6 x 10^9 spermatozoa) or with 2 x 90 mL diluted sperm (total of 6.3 x 10^9 spermatozoa) in the third D5/6 group (IAA).

Data are expressed as means and standard deviations for each treatment. Differences between means have been evaluated by multifactorial analysis in the in vitro experiment (SAS program type II-SS). In the in vivo experiment, differences between means have been analyzed either by Student's *t*-test (litter sizes) or by chi-square test including Yates' correction (percentages of birth) (Pearce, 1965). The motility data were analyzed using the following function in which simple, double and triple interactions were taken into consideration:

$$Y_{ijklm} = \mu + \alpha_i + \beta_{ij} + \gamma_k + \sigma_l + \xi_m + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\alpha\xi)_{im} + (\gamma\delta)_{kl} + (\gamma\xi)_{km} + (\delta\xi)_{lm} + (\alpha\gamma\delta)_{ikl} + (\alpha\gamma\xi)_{ikm} + (\alpha\delta\xi)_{ilm} + (\beta\xi\delta)_{ijkl} + (\beta\delta\xi)_{ijkm} + (\gamma\delta\xi)_{klm} + E_{ijklm}$$

where Y = motility, μ = overall mean, α_i = simple race effect, β_{ij} = within race ejaculate effect, γ_k = diluent effect, σ_l = day effect, ξ_m = incubation effect and E = residue. The day variable was treated as a repetition factor.

RESULTS

Figure 1 shows that the presence of IAA at 10 or 100 ng/mL was without any significant effect over a 13 day period at 15 °C on the percentage of motile spermatozoa (fig 1A) and their motility rate (fig 1B) after 5 min or 3 h of incubation at 39 °C. Although not significant, a slight effect of IAA at a concentration of 10 ng/mL can be observed on both parameters between D7 and D13. In the scope of storing sperm over a 5–6 day period for AI, it is noteworthy that there is almost no decrease in motility parameters between D0 and D5/6 whether IAA is present or not in BTS extender.

Thirty-four ejaculates, each from different boars, were used to study acrosome morphology after 13 days at 15 °C. Table I shows that the presence of IAA (10 ng/mL) in BTS during the 13 day storage leads to a significant increase in the proportion of living spermatozoa with intact acrosome (66 vs 54%; $P < 0.05$).

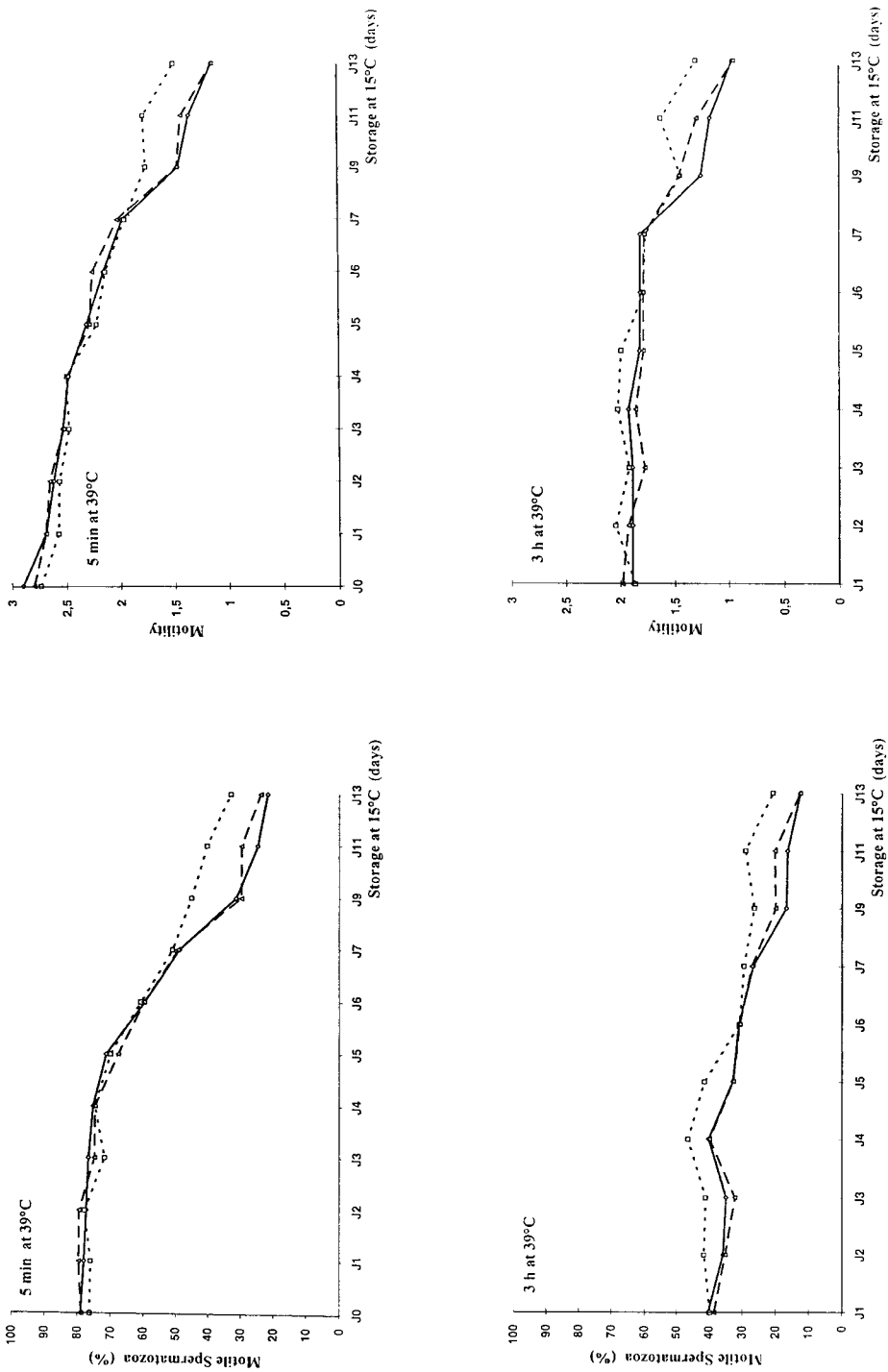

Fig 1. Effect of indole-3-acetic acid (IAA) over a 13 day period on the percentage of motile spermatozoa (left panels) and on motility (right panel) after 5 min (top) or 3 h (bottom) of incubation at 39 °C. Results represent the means for 49 ejaculates. Standard deviations are not shown for the sake of clarity since there is no significative difference between treatments at any day. (◊ — ◊) control (IAA = 0); (□ ··· □) IAA 10 ng/mL; (Δ ---- Δ) IAA 100 ng/mL.

Table I. Acrosome morphology of sperm stored for 13 days in BTS extender in the absence or presence of IAA (10 ng/mL).

Spermatozoa	*Acrosomes*	*BTS alone*	*IAA in BTS*
Living			
	Intact (%)	54 ± 9	66 ± 9
	Altered (%)	7 ± 5	4 ± 2
Dead			
	Total (%)	39 ± 11	30 ± 11

The data are expressed in percent spermatozoa belonging to each class.

The results of the in vivo experiment are shown in table II. There was no significant difference ($P > 0.05$) in fertility and prolificacy between the two groups (A and B) in which the females were inseminated with 6.3 x 10^9 spermatozoa collected the same or previous day (D0/1), whether IAA is present (B) or not (A). Similarly, no difference was observed between groups D (IAA in BTS) and E (BTS alone) in which the females were inseminated with 12.6 x 10^9 spermatozoa stored for 5–6 days at 15 °C. Unexpectedly, the fertility and prolificacy rates with BTS alone at D5/6 (group E) were not

Table II. Fertility and prolificacy rates of sows inseminated with sperm stored for up to 6 days in BTS extender in the absence or presence of IAA (10 ng/mL).

	Conditions			*Results*		
Day *	*Group* (n)	*IAA (ng/mL) in BTS*	*Spz (10^9)*	*Fertility (% farrowing)*	*Prolificacy ** (litter size)*	
					Total	*Alive*
D0/1	A (128)	0	6.3	80.5	11.3 ± 2.9	10.7 ± 2.9
	B (128)	10	6.3	76.6	11.5 ± 3.6	11.1 ± 3.1
D5/6	C (140)	10	6.3	81.4	11.2 ± 3.5	10.6 ± 3.2
	D (132)	10	12.6	80.3	11.4 ± 3.2	10.8 ± 2.9
	E (106)	0	12.6	77.4	11.3 ± 3.1	10.7 ± 2.8

* Storage duration: D0/1 = 0–1 day, D5/6 = 5–6 days; ** mean ± SD. Spz: spermatozoa.

significantly lower than at D0/1 (group A). Nevertheless, the number of sperm used at D5/6 was twice as much as at D0/1. When the sows were inseminated with sperm stored for 5–6 days in the presence of 10 ng IAA per mL of BTS (group C), there was no decrease in fertility and prolificacy even though only 6.3 x 10^9 spermatozoa were used. Because poor results were expected with 6.3 x 10^9 spermatozoa stored for 5–6 days in BTS alone (Paquignon, 1984), such a control group could not be included in the present study.

DISCUSSION

Numerous studies have been carried out to increase the survival duration of boar sperm in liquid form (Bariteau et al, 1977; Slaweta et al, 1981; Paquignon et al, 1987, 1988; Cheng, 1988; Galli and Bosisio, 1988; Rillo et al, 1991; Sone et al, 1992; Galli et al, 1993). The BTS extender is the most largely used extender for boar sperm survival at 15 °C as it allows economically acceptable fertility and prolificacy rates for time periods up to 3 days (Bariteau et al, 1977; Strzezek et al, 1979; Paquignon et al, 1982b, 1987; Galli and Bosisio, 1988; Revell and Glossop, 1989; Sone et al, 1992). Since previous work had shown a beneficial effect of coconut water on sperm survival in different species (Toniolli, 1989, 1990, 1991), we tested here the effects of low concentrations of IAA which was identified as the favourable component from coconut water previously called JYP (Ferreira-Nunès, Combarnous and Leclercq, unpublished data). The effects of IAA during conservation of sperm were studied on motility and morphological parameters in vitro and on fertility and prolificacy parameters in vivo.

Our observation that the percentage of living spermatozoa with undamaged acrosomes after 13 day storage at 15 °C was significantly higher in the presence of IAA (10 ng/mL) compared to control strongly supports an effect of IAA on sperm over this period of time. Nevertheless, there was no evidence that any difference was already present over the initial 5 day period.

Many previous reports have indicated that there is a decrease in boar sperm fertility and prolificacy parameters between 3 and 5 days of conservation in liquid form in various extenders such as BTS, Modena, Kiev, BL1, Androhep and Zorlesco (Johnson et al, 1982, 1988; Weitze, 1990; Machàty et al, 1992; Waberski et al, 1992). Most extenders can be used up to 2 days without any loss in sperm fertilizing capacity (Bariteau et al, 1977; Paquignon et al, 1980) but they can also be used for up to 4 days provided that inseminations are performed with double doses of sperm. Until now, the best results were reported by Machàty et al (1992) who inseminated Large White sows and gilts with 5 x 10^9 sperm stored for 4 days in BTS extender. They obtained a fertility rate of 74.5% and a prolificacy rate of 9.5 live piglets per litter. For sows alone, the figures were 78.1% for fertility and 10.5 for prolificacy, respectively.

The females inseminated with only 6.3 x 10^9 spermatozoa stored for 5–6 days in BTS containing 10 ng/mL IAA (group C) exhibited fertility and prolificacy rates identical to those inseminated with the same number of spermatozoa stored for only 0–1 day in the absence or presence of IAA (groups A and B) as well as to those inseminated with a double number of spermatozoa (12.6 x 10^9) stored for 5–6 days without or with IAA (groups D and E). Thus, our data clearly demonstrate that it is feasible to conserve boar sperm in liquid form for 5–6 days with good results in AI without increasing the number of spermatozoa when IAA is present. This provides additional flexibility and efficiency in breeding technology at AI centers and pig farms since it allows better availability of sperm and its transport to more distant locations.

When we started this experiment, the literature was very pessimistic about the ability of BTS extender alone to fully sustain sperm fertilizing ability for 5–6 days. For this reason, we could not include a control group inseminated with only 6.3 x 10^9 sperm stored for 5–6 days at 15 °C in BTS alone. Consequently, in the present study, we have not positively demonstrated that IAA is responsible for the good sperm fertilizing capacity observed in group C with only 6.3 x 10^9 sperm after 5–6 days at 15 °C since there was no corresponding control group in BTS alone. Nevertheless, data from the literature indicate that the BTS extender provides good results for only up to 4 days using 6 x 10^9 sperm for IA (Johnson et al, 1988; Machàty et al, 1992). A large-scale experiment has been set up to tackle this issue and should provide us with a clear evaluation of IAA effect on boar sperm preservation in liquid form during the year 1996. In parallel, recent data (Blesbois et al, 1996) have also shown a favourable effect of IAA on sperm preservation in the fowl.

It has been known for a long time that the indole-3-acetic acid (IAA) molecule is the main plant hormone named auxin. However, it is only recently that the molecular mechanisms of auxin action in plants have begun to be understood (Barbier-Brygoo, 1995). Auxin binds to a soluble auxin-binding protein and the so-formed complex consecutively associates with a transmembrane receptor which is thought to trigger, directly or indirectly, the various cell responses.

Interaction between the animal epidermal growth factor (EGF) and IAA has been shown to occur and the EGF-IAA complex is more potent than IAA alone on coleoptile growth in various plants (Moon et al, 1994). The reverse situation in which IAA might bind to EGF or another molecule and would potentiate its action in animals thus appears plausible.

It has recently been shown that IAA can accelerate lipid peroxidation catalyzed by haem peroxidase and hydrogen peroxide (Candeias et al, 1995) and such a reaction is expected to have adverse effects on sperm fertilizing ability (Windsor et al, 1993). However, at pH 7.5 this reaction is only observed in the presence of horseradish peroxidase (10 µg/mL) with concentrations of IAA and H_2O_2 of 2.5 x 10^{-4} M and 1 x 10^{-3} M, respectively. In our experimental conditions (no added peroxidase, no H_2O_2 and 5.7 x 10^{-9} M IAA), it is very unlikely that IAA promotes sperm lipid peroxidation.

IAA has also been shown to bind to a glutathion-S-transferase (GST) and to reduce its activity towards its substrates in a noncompetitive manner in plants (Bilang and Sturm, 1995). GST activity has been demonstrated at several levels in the male genital tract: epididymal cells (Robaire and Viger, 1995), in pachytene spermatocytes, spermatids and cytoplasmic droplets (Yoganathan et al, 1989; Papp et al, 1994; Veri et al, 1994) and the µ-class of GST has been detected in mouse and rat spermatozoa (Aravinda et al, 1995; Fulcher et al, 1995). In the rat, GST was detected immunocytochemically on sperm acrosome (Aravinda et al, 1995). Therefore, a possible direct effect of IAA on sperm GST should be taken into consideration to explain its favourable effect on acrosome protection.

In addition, IAA has been demonstrated to accelerate the protein disulfide-isomerase (PDI) activity present in soybean plasma membranes (Morre et al, 1995). PDI activity has also been found at the external surface of platelet plasma membranes (Essex et al, 1995) and hepatocytes (Terada et al, 1995) in mammals and it is known to permit correct refolding of proteins through chaperone-like activity (Song and Wang, 1995). As it is also present in the acrosome of spermatozoa (Ohtani et al, 1993), it might participate in the modifications of acrosome proteins during capacitation. As the main effect of IAA in

boar semen was observed on the integrity of acrosome morphology, it is tempting to hypothesize that it acts by stimulating acrosome PDI.

Is IAA the sole molecule responsible for the protective effect of coconut water in boar sperm preservation? The composition of the liquid endosperm of coconut is very complex and includes various carbohydrates, amino acids and ions, etc. The IAA concentrations used in the present study (10–100 ng/mL) flank the known concentration of active auxin (40 ng/mL) in liquid endosperm of coconut at stages IV–V of development (Dua and Chandra, 1993). Therefore, although other molecules might be involved in the overall effect of coconut water on boar sperm survival, it seems that IAA is responsible for a part in it.

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REFERENCES

Aravinda S, Gopalakrishnan B, Dey CS, Totey SM, Pawshe CH, Salunke D, Kaur K, Shaha C (1995) A testicular protein important for fertility has glutathione-S-transferase activity and is localized extracellularly in the seminiferous tubules. *J Biol Chem* 270, 15675-15685

Barbier-Brygoo H (1995) Tracking auxin receptors using functional approaches. *Crit Rev Plant Sci* 14, 1-25

Bariteau F, Bussière J, Courot M (1977) Insémination artificielle porcine. Amélioration technique, résultats récents. *Jour Rech Porc Fr* 9, 11-14

Bilang J, Sturm A (1995) Cloning and characterization of a glutathione-S-transferase that can be photolabeled with 5-azido-indole-3-acetic acid. *Plant Physiol* 109, 253-260

Blesbois E, Sousa FM, Grasseau I, Combarnous Y (1996) Effects of 3-indole-acetic acid (IAA) on the storage at 4 °C of fowl sperm. *XXth World Poultry Congress,* New Dehli, India

Blichfeldt T, Almlid T, Stavne SE (1988) Liquid preservation of boar semen in Kiev or BTS. A field comparaison. In: *Proceedings of 11th International Congress on Animal Reproduction and Artificial Insemination,* Dublin, Ireland, 229-231

Bork K, Chevrier C, Paquignon M, Jouannet P, Dacheux JL (1988) Analyse de la motilité et du mouvement flagellaire des spermatozoïdes de verrat au cours du transit épididymaire. *Reprod Nutr Dev* 28, 1307-1315

Candeias LP, Folkes LK, Porssa M, Parrick J, Wardman P (1995) Enhancement of lipid peroxidation by indole-3-acetic acid and derivatives: substituent effects. *Free Rad Res* 23, 403-418

Cheng WTK (1988) Preservation of boar semen at 15 °C. *J Chinese Soc Vet Sci* 14, 339-350

Combarnous Y, Ferreira-Nunès J (1995) Sperm extender including indole derivative. International patent n° 2725342; PCT/FR95/01322 (FR 94-12122)

Dua LS, Chandra M (1993) The identification and isolation of plant growth regulating substances from the liquid endosperm of *Cocos nucifera.* In: *Advances in Coconut Research and Development* (MK Nair, HH Khan, P Gopalasundaram, EW Bashkarara, eds), Oxford Publishing Co, Oxford, UK

Essex DW, Chen K, Swiatkowska M (1995) Localization of protein disulfide isomerase to the external surface of the platelet plasma membrane. *Blood* 86, 2168-2173

Freitas VJF, Ferreira-Nunès J (1994) Parâmetros andrologicos y évolucion "in vitro" del semen de ovinos deslanados en la region litoral del nordeste brasileno durante la estacion seca y lluminosa. In: *Proceedings of 7th Jornada Internaccionale de Reproduccion Animal,* Murcia, Spain, 115

Fulcher KD, Welch JE, Klapper DG, O'Brien DA, Eddy EM (1995) Identification of a unique μ-class Glutathione-S-Transferase in mouse spermatogenic cells. *Mol Reprod Dev* 42, 415-424

Galli A, Bosisio M (1988) Quality of semen stored at +15 °/16 °C as related to fertility of artificially inseminated swine. *Theriogenology* 30, 1185-1189

Galli A, Bornaghi V, Balduzzi D, Moretti M (1993) Qualita del semen suino refrigerato à 16 °C. *Suin Ricerca* 1, 55-57

Johnson LA, Aalbers JG, Willems CMT, Rademaker JHM, Rexroad JR (1982) Use of spermatozoa for artificial insemination. III. Fecundity of boar spermatozoa stored in Beltsville liquid and Kiev extenders for three days at 18 °C. *J Anim Sci* 54, 132-136

Johnson LA, Aalbers JG, Grooten JJG (1988) Artificial insemination of swine: fecundity of boar semen stored in Belstsville TS (BTS), modified Modena (MM) or MR-A and inseminated on one, three and four days after collection. *Zuchthyg* 23, 49-55

Kovacs A, Foote RH (1992) Viability and acrosome staining of bull, boar and rabbit spermatozoa. *Biotechnic Histochem* 67, 119-124

Machàty Z, Takacs T, Gathy I (1992) Fertilizing capacity of boar semen diluted with Belstville (BTS) and modified Kiev (MK) extenders in relation to storage time and number of spermatozoa per insemination dose. *Anim Reprod Sci* 29, 289-295

Moon AM, Dyer MI, Brown MR, Crossley DA Jr (1994) Epidermal growth factor interacts with indole-3-acetic acid and promotes coleoptile growth. *Plant Cell Physiol* 35, 1173-1177

Morre DJ, Decabo R, Jacobs E, Morre DM (1995) Auxin-modulated protein disulfide-thio-interchange activity from soybean plasma membranes. *Plant Physiol* 109, 573-578

Ohtani H, Wakui H, Ishino T, Komatsuda A, Miura AB (1993) An isoform of protein disulfide isomerase is expressed in the developing acrosome of spermatids during rat spermiogenesis and is transported into the nucleus of mature spermatids and epididymal spermatozoa. *Histochemistry* 100, 423-429

Papp S, Robaire B, Hermo L (1994) Developmental expression of the glutathione-S-transferase Yo subunit in the rat testis and epididymis using light microscope immunocytochemistry. *Anat Rec* 240, 345-357

Paquignon M (1984) Semen technology in the pig. *Curr Top Vet Med Anim Sci* 30, 202-218

Paquignon M, Bussière J, Bariteau F, Le Maignon De Kerangat G, Courot M (1980) Efficacité des dilueurs Guelph et SCK7 pour la conservation prolongée à l'état liquide du sperme de verrat. *Jour Rech Porc Fr* 12, 157-160

Paquignon M, Bussière J, Bariteau F, Dacheux JL, Courot M (1982a) Effet du dilueur, du taux de dilution et du plasma seminal sur la fertilité des truies après une longue conservation de la semence. *Jour Rech Porc Fr* 14, 85-90

Paquignon M, Dacheux JL, Courot M (1982b) Étude in vitro de quelques facteurs permettant un meilleur maintien de la survie des spermatozoïdes au cours d'une longue conservation. *Jour Rech Porc Fr* 14, 81-84

Paquignon M, Bussière J, Bariteau F (1987) Résultats récents en matière de technologie de la conservation de la semence de verrat. *Jour Rech Porc Fr* 19, 63-78

Paquignon M, Bussière J, Bariteau F (1988) Efficacité des techniques de conservation de la semence de verrat. *INRA Prod Anim* 1, 271-280

Pearce SC (1965) *Biological Statistics: An Introduction.* McGraw-Hill Book Company, New York, 260 p

Reed HCB, Curnock RM (1991) *Proceedings of 2nd International Congress on Boar Semen Preservation.* Paul Parey Publ, Berlin, Germany, 369-373

Revell SG, Glossop CE (1989) A long-time ambient temperature extender for boar semen. *Anim Prod* 48, 579-584

Rillo MS, Sanchez R, Garena Casado P (1991) Current situation of swine artificial insemination technique with liquid semen in Spain. In: *Proceedings of 2nd International Conference on Boar Semen Preservation* 26, 321-324

Robaire B, Viger RS (1995) Regulation of epididymal epithelial cell functions. *Biol Reprod* 52, 226-236

Slaweta R, Sikorskal J, Strzezek J (1981) The effect of storing boar semen at 15 °C for varying lengths of time on the morphology and biological value of spermatozoa. *Medycyna Weterynaryja* 37, 687-690

Sone M, Chikyu M, Yoshida M, Bamba K, Ogasa A (1992) The effect of storing boar semen at 15 °C for varying lengths of time on the morphology and biological value of spermatozoa. *Jpn J Swine Sci* 29, 41-50

Song JL, Wang CC (1995) Chaperone-like activity of protein disulfide isomerase in the refolding of rhodanase. *Eur J Biochem* 231, 312-316

Strzezek J, Smigielska J, Liminowicz J, Czeczot H, Glogowski J (1979) Metabolism of boar semen diluted in different diluents and stored at 18 °–20 °C. *Wojewodzki Zaklad Weterynarii* 153-162

Terada K, Manchikalapudi P, Noiva R, Jauregui, Stockert RJ, Schilsky (1995) Secretion, surface localization, turnover, and steady stage expression of protein disulfide isomerase in rat hepatocytes. *J Biol Chem* 270, 20410-20416

Toniolli R (1989) Estudo das caracteristicas in vitro do sêmen de caprino de raças nativas do nordeste do Brasil, diluido em agua de côco sob a forma in natura, estabilizada e de gel. *Rev Bras Reprod Anim* 13, 209-220

Toniolli R (1990) Avaliacao in vitro do sêmen de suino diluido na agua de côso in natura, establizada e no diluidor BTS. *Rev Bras Reprod Anim* 14, 35-43

Toniolli R (1991) Fertilidade de porcas inseminadas com sêmen diluido em agua de côco estabilizada e com BTS. *Rev Bras Reprod Anim* 15, 133-137

Veri JP, Hermo L, Robaire B (1994) Immunocytochemical localization of glutathione-S-transferase Yo subunit in the rat testis and epidymis. *J Androl* 15, 415-434

Waberski D, Meding S, Dirksen G, Weitze KF, Leiding GC, Willmen T (1992) Fertility results of extender boar semen stored up to five days – a comparison of dilution media. In: *Proceedings of 12th International Congress of Animal Reproduction.* 23-27

Weitze KF (1990) The use of "long-term extender" in pig AI – a view of the international situation. *Pig News Information* 11, 23-26

Windsor DP, White IG, Selley MI, Swan MA (1993) Effects of the lipid peroxidation product (E)-4-hydroxy-2-nonenal on ram sperm function. *J Reprod Fertil* 99, 359-366

Yoganathan T, Eskild W, Hansson V (1989) Investigation of detoxification capacity of rat testicular germ cells and Sertoli cells. *Free Rad Biol Med* 7, 355-359