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Brief communication

Effect of site of sperm deposition on fertility when sows are inseminated with aged semen

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Summary

With conventional insemination, farrowing rate and litter size were lower ($P < .05$) when sperm was aged (≥ 4 days; n = 30) rather than fresh (≤ 3 days; n = 29). Farrowing rate, but not litter size, was maintained with intrauterine insemination of aged sperm (n = 29).

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Resumen - Efecto del sitio de depósito sobre la fertilidad cuando las hembras son inseminadas con semen viejo

Con las inseminación convencional, el porcentaje de fertilidad y el tamaño de la camada fueron menores ($P < .05$) cuando el esperma era viejo (≥ 4 días; n = 30) en vez de fresco (≤ 3 días; n = 29). El porcentaje de fertildiad, pero no el tamaño de la camada, se mantuvo con la inseminación intrauterina de semen viejo (n = 29).

Résumé - Effet du site de déposition du sperme sur la fertilité lors de l'insémination des truies avec de la semence âgée

Lors d'insémination conventionnelle, le taux de mise-bas et la taille des portées étaient plus faibles ($P < .05$) lorsque le sperme était âgé (≥ 4 jours; n = 30) plutôt que frais (≤ 3 jours; n = 29). Le taux de mise-bas, mais pas la taille des portées, était maintenu avec l'insémination intra-utérine de sperme âgé (n = 29).

Conventional artificial insemination (AI) usually involves deposition into the cervix of approximately 2 to 3 × 10^9 sperm in 80 to 100 mL extender. This relatively large number is necessary because most sperm will be lost due to semen backflow and entrapment and death in the cervix and uterus.[1] It is accepted that optimal sow fertility requires sperm deposition within a 24-hour period before ovulation, although the duration of this period is affected by sperm age.[2,3] The optimal period was longer than 24 hours when sows were inseminated with sperm extended in a short-term extender 48 hours or less from collection. However, when sperm age exceeded 48 hours, the optimal period was only 12 hours.[3] The adverse effects of sperm aging are likely mediated by membrane changes associated with increased lipid peroxidation, with the effect of reducing numbers of viable sperm available to form a sperm reservoir.[4,5] In support of this suggestion, when sperm age exceeded 48 hours, farrowing rate was maintained when sperm concentration was doubled.[6]

Catheter designs allowing for intrauterine deposition of sperm reduce sperm losses and permit the initial insemination of fewer sperm while maintaining fertility, presumably because this technique results in the formation of an adequate functional sperm reservoir even with insemination of fewer sperm.[7,8] Subsequent to ovulation, the number of functional sperm available for fertilization will impact sow fertility and is dependent on the number of sperm originally entering the oviductal sperm reservoir, which is influenced by the number of sperm inseminated and their site of deposition. If the effect of increasing sperm age is a reduction in numbers of viable sperm available to enter the sperm reservoir, and given that intrauterine insemination allows normal fertility with fewer sperm, we hypothesized that the adverse effect of increased sperm age on fertility will be countered if aged sperm were deposited into the uterus. The aim of the present study was to compare two different techniques for AI of aged boar sperm.

Materials and methods

The study protocol was approved by the Institutional Animal Care and Use committee of Michigan State University.

The study employed 88 mixed-parity Yorkshire sows housed at the Michigan State University swine facility. Litters were weaned after 24-day lactations and the sows were housed in individual gestation crates. At weaning, sows received an intramuscular injection of 400 IU equine chorionic gonadotrophin and 200 IU human chorionic gonadotrophin (PG600; Intervet Schering-Plough, Millsboro, Delaware). Daily estrus detection commenced 3 days after weaning and involved 2 minutes of fence-line contact with a mature boar and back pressure. Estrus onset was defined as sows exhibiting a standing heat response to back pressure. Sows in estrus on days 4 or 5 after weaning were assigned such as to minimize differences between parity compositions of the groups to conventional cervical AI on the morning of estrus detection and again 24 hours later with 1- and 2-day-old or 2- and 3-day-old semen (Control, n = 29), or cervical AI with 4- and 5-day-old or 5- and 6-day-old semen (Aged-C; n = 30), or intrauterine AI (Deep Golden Pig; IMV, Maple Grove, Minnesota) with 4- and 5-day-old or 5- and 6-day-old semen (Aged-IU; n = 29). Day of semen collection was designated as Day 0. Semen from five Duroc boars was purchased commercially (IBS, Eldora, Iowa) and single sires were equally represented among

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treatments. Semen doses contained 3×10^9 total sperm extended in 80 mL Beltsville Thawing Solution (BTS). Each insemination was performed by one of two technicians. Sperm motility and percent viability were assessed on Days 2, 3, 4, 5, and 6 after collection. Sperm viability and percent normal morphology were evaluated by aniline blue staining of a minimum of 300 sperm and observation under a light microscope at 1000 × magnification.[9]

All data analyses were performed with SAS 9.1 (SAS Institute Inc, Cary, North Carolina). Analysis of variance was used to confirm no difference in mean parity among treatments. Farrowing rates were compared using Fisher's exact test. Litter sizes (total born) were tested using GLM analysis of variance including treatment and parity as main effects in the model. Results are presented as mean ± SE and $P < .05$ was considered significant.

Results

Mean parities were not different among groups (3.3 ± 2.1, 2.5 ± 1.4, and 2.7 ± 1.8 for Control, Aged-C, and Aged-IU, respectively). On Days 2, 3, 4, 5, and 6, values for sperm motility were 83%, 73%, 63%, 63%, and 60%, respectively; for normal morphology, 93%, 94%, 83%, 84%, and 83%, respectively; and for live sperm percent, 94%, 83%, 77%, 74%, and 72%, respectively. Compared to the Control group, the farrowing rate of Aged-C sows was lower ($P < .05$), but the farrowing rate of Aged-IU sows was not different from Control (Table 1). Litter sizes of sows receiving aged sperm were lower ($P < .05$) than for sows receiving fresh sperm regardless of the site of sperm deposition (Table 1).

Table 1: Reproductive performance of sows inseminated cervically with fresh sperm (≤ 3 days old; Control) or inseminated with aged sperm (≥ 4 days old) either cervically (Aged-C) or intrauterine (Aged-IU)*

	Groups of treatment		
	Control (n = 29)	Aged-C (n = 30)	Aged-IU (n = 29)
Farrowing rate (%)	79.3[a]	56.6[b]	72.4[a]
Average parity at farrowing	3.3 ± 2.1	2.5 ± 1.4	2.7 ± 1.8
Total born piglets	13.0 ± 2.3[a]	9.0 ± 3.7[b]	9.8 ± 3.3[b]
Piglets born alive	11.1 ± 2.5[a]	8.0 ± 3.4[b]	8.9 ± 2.9[b]

* Intrauterine catheter: Deep Golden Pig; IMV, Maple Grove, Minnesota.

[ab] Values within a row with different superscripts differ ($P < .05$) as determined using Fisher's exact test (farrowing rate) or ANOVA (litter size).

Discussion

The present results demonstrate a progressive decline of in vitro sperm quality as assessed by motility, morphology, and viability. Although statistical comparisons were not made, when sperm were extended in BTS it appeared that these effects were evident between days 2 and 4 after collection. We have previously demonstrated a link between increasing sperm age and an increase in boar sperm membrane lipid peroxidation and reduced sperm function in vitro.[5,10] Further, in vitro storage time-associated plasma membrane changes have been documented to impair the ability of boar sperm to bind to the oviductal epithelium.[11] This would negatively impact the size of the functional sperm reservoir and potentially reduce sow fertility. It is established that intrauterine sperm deposition allows maintenance of sow fertility with insemination of fewer sperm.[7,8] In the present study, intrauterine deposition of sperm did allow maintenance of farrowing rate, but did not ameliorate the adverse effect of inseminating aged sperm on litter size. It appears that effects on establishment of pregnancy are compensable with increased sperm numbers or deposition deeper into the reproductive tract, while effects on litter size are not compensable. Alternatively, the inability of intrauterine AI to compensate for smaller litters may reflect a need for a larger number of aged sperm, since it has been demonstrated that smaller litters resulted, but without effect on farrowing rate, when very few sperm (0.5×10^9) were inseminated intrauterine.[8]

The nature of the sperm damage that is limiting litter size is unclear, since adverse effects on litter size were noted even with relatively normal in vitro assessed sperm quality on day 5 (> 60% motility and > 70% live sperm). One explanation may be that with increased duration of storage, there is a growing proportion of sperm that are less able to support a normal fertilization. This may involve age-associated increases in plasma-membrane permeability, mitochondrial injury, and possibly DNA damage such as may result from membrane peroxidation.[4,5] It is possible that aged sperm are capable of fertilizing ova, but the resulting embryos are subject to increased mortality, resulting in smaller litter sizes.[12] An ability to effectively compete for fertilization but an increased risk of embryos failing to develop normally could explain why the effect of aged sperm on litter size is non-compensable.

Implications

- Conventional insemination of aged sperm adversely affects sow reproductive performance.
- Under the conditions of this study, intrauterine insemination of aged sperm permits normal farrowing rates but still results in smaller litter sizes.

References

1. Steverink DWB, Soede NM, Bouwman EG, Kemp B. Semen backflow after insemination and its effects on fertilisation in sows. *Anim Reprod Sci*. 1998;54:109–119.

2. Nissen AK, Soede NM, Hyttel P, Schmidt M, D'Hoore L. The influence of time of insemination relative to time of ovulation on farrowing frequency and litter size in sows, as investigated by ultrasonography. *Theriogenology*. 1997;47:1571–1582.

3. Waberski D, Weitze KF, Lietmann C, Lubbert zur Lage W, Bortolozzo FP, Willmen T, Petzoldt R. The initial fertilizing capacity of longterm-stored liquid boar semen following pre- and postovulatory insemination. *Theriogenology*. 1994;41:1367–1377.

4. Kumaresan A, Kadirvel G, Bujarbarbaruah KM, Bardoloi RK, Das A, Kumar S, Naskar S. Preservation of boar semen at 18°C induced lipid peroxidation and apoptosis like changes in spermatozoa. *Anim Reprod Sci*. 2009;110:162–171.

5. Am-in N, Kirkwood RN, Techakumphu M, Tantasuparuk W. Effect of storage for 24 h at 18°C on sperm quality and a comparison of two assays for sperm membrane lipid peroxidation. *Can J Anim Sci*. 2010;90:389–392.

6. Johnson LA, Aalbers JG, Grooten HJG. Artificial insemination of swine: Fecundity of boar semen stored in Beltsville TS (BTS), Modified Modena (MM), or MR-A and inseminated on one, three and four days after collection. *Zuchthygiene.* 1988;23:49–55.

7. Watson PF, Behan JR. Intrauterine insemination of sows with reduced sperm numbers: results of a commercially based field trial. *Theriogenology*. 2002;57:1683–1693.

8. Rozeboom KJ, Reicks DL, Wilson ME. The reproductive performance and factors affecting on-farm application of low-dose intrauterine deposit of semen in sows. *J Anim Sci*. 2004;82:2164–2168.

9. Borga K, Colenbrander B, Fazeli A, Parlevliet J, Malmgren L. Influence of thawing method on motility, plasma membrane integrity and morphology of frozen-thawed stallion spermatozoa. *Theriogenology*. 1997;48:531–536.

10. Am-in N, Kirkwood RN, Techakumphu M, Tantasuparuk W. Lipid profiles of sperm and seminal plasma from boars having normal or low sperm motility. *Theriogenology*. doi:10.1016/j.theriogenology.2010.10.032.

11. Waberski D, Magnus F, Ardon F, Petrunkina AM, Weitze KF, Topfer-Petersen E. Binding of boar spermatozoa to oviductal epithelium in vitro in relation to sperm morphology and storage time. *Reproduction*. 2006;131:311–318.

12. First NL, Stratman FW, Casida LE. Effect of sperm age on embryo survival in swine. *J Anim Sci*. 1963;22:135–138.