Fertility after intrauterine insemination with conventional or low numbers of spermatozoa in sows with synchronized ovulation

Christine Pelland, MSc; Glen Cassar, DVM, PhD; Roy Kirkwood, DVM, PhD, Diplomate ECAR; Robert Friendship, DVM, MSc, Diplomate ABVP

Summary

Objective: To determine sow fertility to a single timed intracervical or intrauterine insemination of conventional or low sperm numbers.

Materials and methods: A total of 411 mixed-parity sows were subjected to controlled ovulation by injection of 600 IU equine chorionic gonadotrophin at weaning and 5 mg porcine luteinizing hormone (pLH) 80 hours later. Sows were assigned to a single insemination of 1 or 3×10^9 sperm delivered into either the cervix or uterus. Inseminations were performed approximately 36 hours after pLH injection. Intensity of standing estrus at insemination was subjectively scored as 1 to 3, with 3 being a stronger response, and semen backflow was recorded as yes or no.

Results: Number of sperm and site of deposition did not affect pregnancy or farrowing rates or subsequent litter size. Mean farrowing rates were 68.32% and 68.63% in sows inseminated using an intrauterine catheter and either 1 or 3×10^9 sperm, respectively. In sows inseminated using the cervical method, farrowing rates were 77.88% and 67.31% when 1 and 3×10^9 sperm were used, respectively. Greater intensity of estrus at insemination was associated with higher pregnancy and farrowing rates ($P < .001$), and backflow during insemination was associated with lower pregnancy and farrowing rates ($P < .01$).

Implications: When appropriately timed after induced ovulation, insemination of low sperm numbers does not adversely affect sow fertility, and this lack of effect is independent of the site of sperm deposition.

Keywords: swine, intrauterine insemination, controlled ovulation

Received: August 28, 2007
Accepted: February 5, 2007

Resumen - Porcentaje de fertilidad obtenido después de la inseminación intrauterina con un número bajo o convencional de espermatozoides en hembras con ovulación sincronizada

Objetivo: Determinar la fertilidad de las hembras con una inseminación única intrauterina o intracervical, programada y con un número bajo o convencional de espermatozoides.

Materiales y métodos: Un total de 411 hembras de paridad mixta se sometieron a una ovulación controlada con una inyección de 600 IU de gonadotropina coriónica equina al destete y 5 mg de hormona luteinizante porcina (pLH por sus siglas en inglés) 80 horas después. Las hembras se asignaron a una inseminación única conteniendo ya sea 1 ó 3×10^9 espermatozoides depositados en el cérvix o en el útero. Las inseminaciones se realizaron aproximadamente 36 horas después de la inyección de pLH. La intensidad del estro en el momento de la inseminación recibió un puntaje subjetivo de 1 a 3, siendo 3 la respuesta más fuerte, y el contra flujo de semen se registró como si ó no.

Resultados: El número de espermatozoides y el sitio de depósito no afectaron la gestación o el porcentaje de fertilidad o el subsiguiente tamaño de camada. El porcentaje de fertilidad fue de 68.32% y 68.63% en hembras inseminadas utilizando un catéter intrauterino y una cantidad de espermatozoides, de 1 ó 3×10^9, respectivamente. En hembras inseminadas utilizando el método cervical, el porcentaje de fertilidad fue de 77.88% y 67.31% cuando se utilizó 1 y 3×10^9 espermatozoides, respectivamente. Una mayor intensidad de estro en la inseminación se asoció con índices más altos de gestación y fertilidad ($P < .001$), y el contra flujo durante la inseminación se asoció con porcentajes más bajos de gestación y fertilidad ($P < .01$).

Implicaciones: Cuando la inseminación con bajo número de espermatozoides se programa correctamente, después de la ovulación inducida, esta no afecta negativamente la fertilidad de la hembra, y esta falta de efecto es independiente del sitio en el que se depositen los espermatozoides.

Résumé - Fertilité après insémination intra-utérine avec un nombre conventionnel ou réduit de spermatozoïdes chez des truies avec ovulation synchronisée

Objectif: Déterminer la fertilité chez des truies suite à une insémination intracervicale ou intra-utérine unique calculée d'un nombre conventionnel ou réduit de spermatozoïdes.

Matériels et méthodes: Un total de 411 truies de parité mixte ont été soumises à une ovulation contrôlée en leur injectant 600 UI de gonadotrophine chorionique équine lors du sevrage et 5 mg d'hormone lutéinisante porcine (pLH) 80 heures plus tard. Les truies ont été inséminées

CP, GC, RF: Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada

RK: Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan.

Corresponding author: Dr Robert Friendship, Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada N1G 2W1; E-mail: rfriends@uoguelph.ca.

This article is available online at http://www.aasv.org/shap.html.

Pelland C, Cassar G, Kirkwood R, et al. Fertility after intrauterine insemination with conventional or low numbers of spermatozoa in sows with synchronized ovulation. *J Swine Health Prod.* 2008;16(4):188–192.

avec une dose unique de 1 ou 3×10^9 spermatozoïdes déposés soit dans le col utérin ou l'utérus. Les inséminations ont été effectuées environ 36 heures après l'injection de pLH. L'intensité de la chaleur au moment de l'insémination était mesuré subjectivement par un pointage de 1 à 3, 3 étant la réponse la plus forte, et le reflux de semence noté comme oui ou non.

Résultats: Le nombre de spermatozoïdes et le site de déposition n'ont pas affecté les taux de gestation ou de mise-bas, de même que la taille des portées. Les taux moyens de mise-bas étaient de 68.32% et 68.63% chez les truies inséminées en utilisant un cathéter intra-utérin avec, respectivement, 1 ou 3×10^9 spermatozoïdes. Chez les truies inséminées au niveau du col utérin, les taux de mise-bas étaient respectivement de 77.88% et 67.31% lorsque 1 et 3×10^9 spermatozoïdes étaient utilisés. Une intensité plus grande de l'œstrus au moment de l'insémination était associée avec des taux de gestation et de mise-bas plus élevés ($P < .001$), et le reflux lors de l'insémination était associé avec des taux plus faibles de gestation et de mise-bas ($P < .01$).

Implications: Lorsque calculée de manière appropriée après l'induction de l'ovulation, l'insémination d'un nombre réduit de spermatozoïdes n'a pas affecté de manière adverse la fertilité des truies, et cette absence d'effet est indépendante du site de déposition de la semence.

Although artificial insemination (AI) of sows has been established for many decades, the use of boar ejaculates remains inefficient, in that relatively large numbers of sperm are inseminated multiple times during estrus. Early research suggested that 5 to 10×10^9 sperm in 100 mL extender was necessary to achieve optimal fertility.[1] Since then, numbers of sperm per dose have decreased, but 2 to 4×10^9 sperm in 80 to 100 mL extender are still currently considered necessary for optimum fertility.[2] Improved efficiency of use of boar ejaculates requires insemination of fewer sperm. The number of sperm inseminated per service may be reduced either by reducing the number of sperm per insemination dose or the number of inseminations per service.

Adoption of new techniques involving insemination of low sperm concentrations requires optimization of insemination protocols.[3,4] Martinez et al[3] nonsurgically deposited sperm two-thirds of the way along one uterine horn, a procedure referred to as deep intrauterine insemination. They reported that in sows in which ovulation was synchronized and deep intrauterine insemination used with as few as 50×10^6 sperm, fertility was comparable to that of nonsynchronized sows inseminated conventionally with 3×10^9 sperm. If sperm are surgically placed at the uterotubal junction, as few as 10×10^6 sperm are required.[5,6] This illustrates that fewer sperm are required for normal sow fertility with progressively deeper sites of sperm deposition. Insemination catheters that deposit sperm beyond the cervix into the uterine body are commercially available. Sow fertility was maintained[7] when semen doses containing only 1×10^9 sperm were deposited transcervically (ie, intrauterine insemination), although multiple inseminations were still employed.

It has been established that for optimal sow fertility, sperm must be deposited within 24 hours before ovulation,[8] but that if intrauterine inseminations of fewer sperm are performed, then timing of insemination relative to ovulation becomes more important.[9] If the time of ovulation is known, then a single insemination should suffice for optimal fertility. In order to obtain good predictability of the time of ovulation, pharmaceutical control is required. A protocol that uses equine chorionic gonadotrophin (eCG) for induction of a synchronous estrus, followed by porcine luteinizing hormone (pLH) for accurate timing of ovulation, has been successful.[10] Further, subsequent to a controlled estrus and ovulation, sow fertility after a single insemination was comparable to that after double inseminations.[9,10] The cost of the exogenous hormones can be offset by the reduction in labor needed for estrus detection and the reduced semen costs associated with only one insemination per service. The present study was undertaken to test the hypothesis that sow fertility will be maintained if fewer sperm are deposited transcervically close to the time of ovulation.

Materials and methods

This study was conducted from February to October 2005 in a commercial 350-sow farrow-to-finish purebred pig operation. The University of Guelph Animal Care Committee approved the experimental protocol. Breeding records for the previous 3 years were examined before the study began. Farrowing rate averaged approximately 70% each year.

Each week at weaning, each sow was randomly assigned to one of four treatment groups. A total of 411 mixed-parity purebred Yorkshire (n = 248), Landrace (n = 119), and Duroc sows (n = 44) each received an intramuscular (IM) injection of 600 IU eCG (Pregnecol 5000; Bioniche Animal Health, Belleville, Ontario, Canada) at the time of weaning to induce synchronous ovarian follicular development and estrus. At 80 hours after the eCG injection, sows received an IM injection of 5 mg pLH (Lutropin-V; Bioniche Animal Health) to induce ovulation. The predicted time of ovulation was 38 hours after pLH injection.[10,11] Sows were randomly assigned by parity and breed to treatment in a 2×2 factorial arrangement. The main effects were site of sperm deposition (cervical or intrauterine) and number of sperm inseminated (1 or 3×10^9). Treatment groups are described in Table 1. All semen doses were extended to 80 mL in Androhep (Minitube Canada, Ingersol, Ontario, Canada). Sows were inseminated 36 hours after pLH injection, within 48 hours of semen collection. Intrauterine and cervical insemination catheters (both foam tipped) and Androhep semen extender were supplied by Minitube Canada.

Semen was supplied by Total Swine Genetics (Tillsonburg, Ontario, Canada), with sire selected for each sow by the herdsman, who was blinded to sow treatment group. Duplicate samples of each semen dose were prepared and evaluated for numbers of live sperm per dose (concentration and motility) and progressive motility using a computer-assisted semen-analysis machine at the University of Guelph (Sperm Vision; Minitube Canada).

During insemination, the intensity of estrus behavior was subjectively scored by the breeding technician (1 to 3, with 3 the most intense behavior), and occurrence of semen leakage during insemination was noted. The volume of lost semen was not measured. Pregnancy status was determined 28 days post breeding by real-time ultrasonography (Agroscan, Angouleme, France). Treatment effects were assessed on the basis of pregnancy and farrowing rates and subsequent total born litter size. Three different technicians performed the inseminations over the course of the project.

Table 1: Conception rate, farrowing rate, and total born litter size of sows inseminated with either a cervical or intrauterine catheter and either 1 or 3×10^9 sperm per dose*

	Cervical catheter		Intrauterine catheter	
	Group A	**Group B**	**Group C**	**Group D**
Sperm dose	1×10^9	3×10^9	1×10^9	3×10^9
No. of sows	104	104	101	102
Conception rate (%)	80.77	74.04	73.27	78.43
Farrowing rate (%)	77.88	67.31	68.32	68.63
Total litter size†	10.30 ± 3.51	10.93 ± 3.08	10.79 ± 2.83	10.27 ± 3.27

* Conception rate (chi-square; $P = .53$), farrowing rate (chi-square; $P = .30$), and litter size (two-way ANOVA; $P = .67$) did not differ among the four treatment groups.

† Mean ± SE.

The efficacy of the ovulation synchronization protocol was confirmed in 14 sows enrolled in the trial by transrectal real-time ultrasound (RTU) examination of their ovaries using an Aloka SSD 500 (Aloka, Vaudreuil-Dorion, Quebec, Canada) with a 7.5-MHz linear array transducer for visualization of the ovaries.[12] These sows were a convenience sample selected when equipment was available. The purpose of this small ultrasound study was to reassure the herdsman that the induction protocol resulted in synchronized ovulation in this herd as described in published reports.[10] Examinations were performed 1 hour prior to pLH injection, then at 13, 22, 36, 46, and 60 hours after pLH injection. Ovulation was considered to be complete when there were fewer than four follicles > 6.5-mm diameter remaining on the ovaries.[13]

Statistical analysis

The calculated sample size (number of sows per treatment group) was approximately 100, with 80% power and 95% confidence to show a difference in litter size of one pig per litter, assuming a standard deviation of two pigs per litter. This sample size would be sufficient to calculate a difference in farrowing rate of approximately 18% (Stata 9; StataCorp, College Station, Texas). Descriptive statistics (eg, means, standard deviations, and proportions) were conducted in the Statistix7 program (Analytical Software 2000, Tallahassee, Florida). Data were analyzed using the Statistical Analysis System package version 8.2 (SAS Institute Inc, Cary, North Carolina). Univariant models were used to assess the effect of catheter type and sperm number per dose on fertility parameters. Treatment effects on conception rate and farrowing rate were analysed using chi-square tests. The association between estrus status or observed semen leakage and conception rate and farrowing rate were tested using a Spearman correlation coefficient. Association between type of catheter or sperm concentration and litter size was tested using a two-way analysis of variance (ANOVA). The interaction between treatments after adjusting for observed backflow, sow parity, and semen motility as fixed effects, and boar and breed as random effects, was tested with a linear mixed model. This model was evaluated using tests of normality and equal variances and fit all criteria. Results are presented as mean ± SE, and $P < .05$ was considered statistically significant.

Results

Ovarian RTU examinations revealed that no sow had ovulated earlier than 36 hours after pLH injection, one sow was ovulating at 36 hours, and all sows had completed ovulation by 46 hours. There were no significant effects of treatment on conception rate, farrowing rate, or subsequent total litter size (Table 1). However, estrus status of the sows at the time of insemination was associated both with conception rate and farrowing rate (Table 2). As the score for estrus status at the time of insemination increased, the more likely the sow was to conceive and farrow. Semen leakage was also related to conception and farrowing rates (Table 3). Occurrence of leakage at insemination decreased the likelihood that a sow would conceive and subsequently farrow.

The intrauterine catheter was relatively easy to use, although there were some difficulties in passing the inner catheter into the uterine body of a few sows (generally the youngest animals). In this study, the catheter was inserted to the point of no resistance (the uterine body). If too much resistance was encountered, the catheter was left in the sow's cervix for a few minutes before proceeding. In most cases, it then passed through the remainder of the cervix into the uterine body with no further difficulty. In the few sows where this was not the case, the catheter was gently advanced as far as possible (though not to the full length) and insemination was performed at that point. There appeared to be no difference in conception rate among sows inseminated by different breeding technicians.

The average parity in this herd during the study period was 3.17.

Discussion

Previous studies have shown that conventional cervical insemination of relatively low numbers of sperm (1×10^9 compared to the conventional 3×10^9 sperm) resulted in lower farrowing rates and smaller litters, an effect that was prevented by intrauterine insemination.[7] Others have reported that maintenance of sow fertility subsequent to low-dose insemination was affected by the wean-to-estrus interval,[14] supporting the suggestion that as numbers of sperm inseminated decrease, the accuracy of timing of sperm deposition relative to ovulation becomes more important.[9] In the present study, we observed no difference in sow fertility associated with insemination of fewer sperm, regardless of whether deposition was cervical or intrauterine.

Table 2: Conception and farrowing rates of sows evaluated at the time of insemination for intensity of estrus behavior*

	Score of estrus intensity			*P*†
	1	2	3	
No. of sows	10	42	148	NA
Conception rate (%)	20	60	81	< .001
Farrowing rate (%)	20	60	76	< .001

* Scored as 1 to 3, with 3 the most intense behavior.

† Spearman correlation coefficient. NA = not applicable.

Table 3: Conception and farrowing rates of sows when semen leakage either was or was not observed at the time of insemination using either intracervical or intrauterine insemination

	Leakage	No leakage	*P**
No. of sows	47	364	NA
Conception rate (%)	57	79	.001
Farrowing rate (%)	55	73	.01

* Spearman correlation coefficient. NA = not applicable.

The lack of detrimental effect after conventional insemination of only 1×10^9 sperm was unexpected and likely resulted from optimal timing of insemination relative to ovulation. The ability to accurately predict the time of ovulation, and so optimize the timing of insemination, was presumably a function of our control of the time of ovulation with pLH. Administration of gonadotrophins such as eCG to induce estrus is associated with a longer interval between estrus detection and ovulation.[13,15] In sows expected to ovulate 40 hours or more after estrus detection (late-ovulating sows), controlling the time of ovulation becomes relatively simple, since injection of pLH will advance ovulation to an average of 38 hours after the pLH injection.[10,11] Indeed, in the present study, all sows examined with RTU had ovulated within 8 hours after insemination. The objective of the insemination, regardless of numbers of sperm originally deposited, site of deposition, or timing of insemination, is to achieve a sufficient number of sperm in the oviductal sperm reservoir at the time of ovulation to ensure optimal fertilization. If timing of insemination is less than optimal, then insemination of larger numbers of sperm, either as a single insemination or multiple inseminations, will likely be necessary. In contrast, results of the present study indicate that sow fertility is maintained even with conventional insemination of a lower than conventional number of sperm, as long as insemination occurs close to the time of ovulation.

Estrus score is routinely used as a behavioral indicator of when to breed a sow.[9] It is generally accepted that when a sow is first observed in standing estrus, she is likely to ovulate within 24 to 48 hours. In the present study, sows were bred at a fixed time regardless of their estrus behaviour. It was believed that the injection of pLH would ensure that all sows would ovulate within 8 hours post insemination, regardless of estrus status. However, the association between intensity of estrus behavior and sow fertility in this study indicates that factors in addition to timing of insemination are involved in reproductive outcomes. It is possible that in sows exhibiting a less intense behavioral estrus, uterine contractility is less intense, which might result in poorer transport of sperm from the site of deposition to the uterotubal junction and the sperm reservoir, and so adversely affect fertility.

In this study, semen backflow was observed during both cervical and intrauterine inseminations. Although evidence of semen backflow has been associated with reduced sow fertility, this adverse effect was noted only for sows receiving low numbers of sperm.[16,17] The use of intrauterine catheters has been suggested as a means of reducing the amount of backflow during insemination.[17] However, in our study, the number of sows in which semen backflow occurred was similar for both catheter types. This agrees with the results of Mezalira et al,[18] who stated that backflow during insemination is a common occurrence in sows, independent of the site of semen deposition. Stimulation of uterine contractility during insemination can negatively affect sperm transport and fertility by increasing the amount of leakage and backflow observed.[17] The use of an intrauterine catheter, compared to the standard cervical catheter, is an additional cervical stimulus and therefore may cause greater uterine contractility and a greater risk of backflow or leakage. The leakage associated with use of intrauterine catheters in this study may have masked the superior conception rate previously reported[5] for this type of catheter over standard cervical catheters when fewer sperm are inseminated. These results show that backflow at the time of insemination decreases the sow's ability to conceive, independent of the type of catheter or sperm concentration used.

It has been noted previously that intrauterine catheter placement requires more time than placement of cervical catheters.[19] Watson and Behan[7] stated that a proportion of animals have pre-existing lesions in the reproductive tract which predispose them to injury if the catheter is forced beyond the point of resistance. They also stated that there is no disadvantage if the device cannot be introduced to its full depth. However, in the present study, difficulty with proper placement of the intrauterine catheter in some sows perhaps decreased its effectiveness, in comparison with cervical catheters, when low numbers of sperm were inseminated. Intrauterine catheters proved to be practical, but of no economic advantage in this study. Further work needs to be done to assess whether conception rates would differ between cervical and intrauterine insemination methods if even lower numbers of sperm were used.

In addition to observations at the time of insemination (leakage and estrus status), other factors must be considered when a sow's reproductive performance is assessed.

Realistic targets of productivity for individual herds must take into account the genotype, parity distribution, and disease level of the animals, as well as management practices and housing. All of these factors affect the number of piglets born alive and resulting litter size.[20]

Due to multiple factors affecting a sow's reproductive performance, blinded randomization of the sows to treatment was performed to ensure that parity and breeds were evenly distributed among treatment groups. It was concluded that there was no effect of sow breed or sow parity on litter size.

Implications

- When sows are inseminated with fewer sperm close to the time of predicted ovulation, fertility is maintained even when sperm are deposited into the cervix.
- Insemination of sows expressing relatively weak estrus signs likely results in poorer fertility.
- Intrauterine insemination does not prevent semen leakage.
- Under the conditions of this study, semen leakage is associated with poorer fertility regardless of original sperm dose or site of deposition.

Acknowledgements

We gratefully acknowledge the financial support of Ontario Pork and the OMAF-University of Guelph Animal Research Program. Hormones were generously provided by Bioniche Animal Health, and artificial insemination materials were supplied by Minitube Canada.

References

1. Baker RD, Dziuk PJ, Norton HW. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. *J Anim Sci.* 1968;27:88–93.

*2. Colenbrander B. Commercial use of swine AI world-wide: a roundtable. *Reprod Dom Anim.* 1991;Suppl1:298–333.

3. Martinez EA, Vazquez JM, Roca J, Lucas X, Gil MA, Parrilla I, Vazquez JL, Day BN. Minimum number of spermatozoa required for normal fertility after deep intrauterine insemination in non-sedated sows. *Reproduction.* 2002;123:163–170.

4. Rath D. Low dose insemination in the sow – A review. *Reprod Dom Anim*. 2002;37:201–205.

5. Krueger C, Rath D, Johnson LA. Low dose insemination in synchronized gilts. *Theriogenology*. 1999;52:1363–1373.

6. Krueger C, Rath D. Intrauterine insemination in sows with reduced sperm number. *Reprod Fertil Dev*. 2000;17:113–117.

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. 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.

9. Garcia JC, Abad M, Kirkwood RN. Effect of sperm numbers and time of insemination relative to ovulation on sow fertility. *Anim Reprod Sci*. 2007;100:397–401.

10. Cassar G, Kirkwood RN, Poljak Z, Bennett-Steward K, Friendship RM. Effect of single or double insemination on fertility of sows bred at an induced estrus and ovulation. *J Swine Health Prod.* 2005;13:254–258.

11. Abad M, Garcia JC, Sprecher DJ, Cassar G, Friendship RM, Buhr M, Kirkwood RN. Effect of insemination-ovulation interval and addition of seminal plasma on sow fertility to insemination of cryopreserved sperm. *Reprod Dom Anim*. 2007;42:418–422.

12. Knox RV, Althouse GC. Visualizing the reproductive tract of the female pig using real-time ultrasonography. *Swine Health Prod*. 1999;7:207–215.

13. Knox RV, Rodriguez-Zas SL, Miller GM, Willenburg KL, Robb JA. Administration of P.G.600 to sows at weaning and the time of ovulation as determined by transrectal ultrasound. *J Anim Sci*. 2001;79:796–802.

14. 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.

15. Lucia T Jr, Correa MN, Deschamps JC, Peruzzo IA, Matheus JEM, Aleixo JAG. Influence of equine chorionic gonadotropin on weaning-to-estrus interval and estrus duration in early-weaned, primiparous, female swine. *J Anim Sci*. 1999;77:3163–3167.

16. Steverink DWB, Soede NM, Bouwman EG, Kemp B. Semen backflow after insemination and its effects on fertilisation in sows. *Anim Reprod Sci*. 1999;54:109–119.

*17. Levis DG, Burroughs S, Williams S. Use of intra-uterine insemination of pigs: Pros, cons & economics. Ohio Pork Center. The Ohio State University Extension. 2005. Available at: **http://porkinfo.osu.edu/Word%20Documents/AIintrauterineDL.doc**. Accessed 14 May 2008.

18. Mezalira A, Dallanora D, Bernardi ML, Wentz I, Bortolozzo FP. Influence of sperm cell dose and post-insemination backflow on reproductive performance of intrauterine inseminated sows. *Reprod Dom Anim*. 2005;40:1–5.

*19. Belstra BA. Review: Intrauterine (transcervical) and fixed-time artificial insemination in swine. North Carolina State University Annual Swine Report 2002. Available at: **http://mark.asci.ncsu.edu/SwineReports/2002/belstra3.htm**. Accessed 27 April 2008.

*20. Aherne F, Kirkwood R. Alberta Pork Research. Factors affecting litter size. Available at: **http://www.thepigsite.com/articles/2/ai_genetics_reproduction/304/factors_affecting_litter-size**. Accessed 14 March 2008.

* Non-refereed references.