Evaluation of the hypothetic suitability of using oestrogens and oxytocin as a semen additive to reduce the time required for the completion of pig artificial insemination

J. PELÁEZ*, J. A. RIOL², B. ALEGRE¹, F. J. PEÑA¹ and J. C. DOMÍNGUEZ¹

¹ Department of Animal Pathology (Animal Health). Unit of Reproduction and Obstetrics. Veterinary Faculty. University of León. Campus de Vegazana, 24071 León (Spain).
² Department of Animal Production II.
*Corresponding author.

SUMMARY

The aim of this study was to ascertain whether oestrogens and oxytocin are useful as seminal additives to reduce the time of semen uptake during pig artificial insemination - an indicator of labour demands for this activity -, so that higher reproductive performances can be obtained when evaluated as the number of piglets produced on a per time of labour basis. Adult sows were inseminated twice with a 24 h-interval using additive-enriched semen (n = 43) or non-treated semen (n = 41). The time of semen uptake in each insemination was recorded, and the number of piglets produced on a per time of labour basis calculated. The use of additive only reduced semen absorption time in the first artificial insemination (1.55 ± 1.06 vs. 2.21 ± 1.53 minutes; P<0.05), and although litter size was not significantly improved by using treated semen, the number of piglets produced on a per time of labour basis did significantly increase in this group of sows (3.27 ± 1.33 vs. 2.54 ± 1.30; P<0.05). Results suggest that the beneficial effect of oestrogens and oxytocin on reducing labour demands was only noticed in the first insemination, but this can account for the achievement of better reproductive performances on a per time of labour basis.

Keywords: pig - artificial insemination - reproductive performance - uterine motility - oestrogens - oxytocin.

RÉSUMÉ

Evaluation de l’intérêt d’une addition d’oestrogènes et d’ocytocine dans le sperme porcin pour réduire le temps passé à la réalisation d’inséminations artificielles. Par J. PELÁEZ, J. A. RIOL, B. ALEGRE, F. J. PEÑA et J. C. DOMÍNGUEZ.

L’objectif de cette étude était de vérifier si l’addition d’oestrogènes et d’ocytocine dans le sperme porcin lors d’inséminations artificielles permettait de réduire le temps d’absorption de la semence - un indicateur du temps de travail requis pour cette activité. Le but était d’améliorer les performances en reproduction en terme de nombre de porcelets produits par unité de temps de travail. Des truies adultes ont été inséminées deux fois à 24 heures d’intervalle en utilisant de la semence avec additifs (n = 43) ou de la semence sans additif (n = 41). Le temps d’absorption de la semence pour chaque insémination a été mesuré, et le nombre de porcelets produits par unité de temps de travail a été calculé. L’utilisation des additifs a permis de réduire uniquement le temps d’absorption lors de la première insémination (1.55 ± 1.06 vs. 2.21 ± 1.53 minutes ; P<0.05). Bien que la taille de la portée n’ait pas été augmentée significativement lors de l’utilisation d’additifs dans la semence, le nombre de porcelets produits par unité de temps de travail a été calculé. L’utilisation des additifs a permis de réduire la semence et de l’ocytocine pour réduire le temps de travail n’a donc été observé que lors de la première insémination artificielle, mais cet effet peut permettre d’améliorer les performances en reproduction par unité de temps de travail.


Introduction

Artificial insemination (A.I.) in swine herds has several advantages over natural mating: apart from the traditional sanitary, economic and zootechnical benefits, it may also reduce labour requirements [4]. Comparative labour studies between on-farm A.I. and natural mating have shown that whenever four or more gilts or sows need to be bred on a single day, A.I. significantly reduces the amount of time required [7]. In large herds batch weaning procedures are routinely performed and a considerable number of sows then have to be inseminated [20]. Therefore, this labour-saving approach of A.I. may be of special interest. Moreover, optimization of the breeding management in pigs involves maximizing reproductive performance, which, for its part, needs to be evaluated in conjunction with some production expenditures such as labour requirements. In such a scenario, the number of pigs produced on a per time of labour basis becomes a valid expression for studying the reproductive performances of any farm [8]. Thus, efforts to reduce labour requirements at the time of breeding should not be neglected, since they might become valuable tools for the improvement of the piggery’s reproductive performance.

On-farm A.I. regimens in pig units involve devoting time for oestrus detection, semen collection and processing, insemination procedures, and equipment cleaning as the most important labour demands. Among these different stages, the uptake of inseminate does not seem to be particularly time-consuming or of a concern [7]. However, in these authors’ opinion, there could certainly be further advantages in terms of labour-saving if an increased myometrial activity during insemination led to a faster absorption of the semen.

Stimulation of myometrial activity during insemination can be achieved in two different ways: by simply setting the
catheter of insemination (cervical stimulation), and by performing the inseminations in the presence of a teaser boar. Cervical stimulation is thought to enhance uterine activity through an adrenergic or cholinergic pathway, whereas the boar presence acts through a release of oxytocin [15]. For the first method to be effective, some authors [16] suggest postponing the insemination for two minutes after setting the catheter, which obviously would not represent a method of choice for reducing labour requirements. The second possibility may be of little value if the inseminations need to be conducted at different locations in the piggery, or the boar management/behaviour is particularly awkward. In contrast, the addition of oxytocin (O.T.) and/or oestrogens (O.E.) to semen might be an alternative, as both substances could affect myometrial contractility when administered directly into the uterus lumen [12, 22]. This practice has been mainly used for enhancing sows’ reproductive performance, leading to variably significant results [3, 5, 10, 11, 18, 25], but no data seem to exist concerning the profitability of the effect of these substances on uterine contractions in the reduction of A.I. labour demands. We therefore performed an investigation to ascertain whether the strategy of adding both O.E. and O.T. to the inseminate successfully reduces semen absorption time when a hands-free insemination method is used. The hypothetic suitability of this procedure for attaining better reproductive performances while reducing semen absorption time was then evaluated.

Materials and methods

The study was conducted during a one month-long period in a normal fertility season (from mid-October to mid-November) [6], in an intensive piggery housing 932 sows where A.I. is practiced systematically twice per week upon batches of gilts and weaned sows with an average of 12 animals. Crossbred pigs used in this experiment were Landrace x Yorkshire, which were individually penned from weaning to day 110 of gestation.

SEmen Collection, Oestrus Detection AND Insemination Procedure

Semen was collected from 9 mature hybrid boars (Genética Hypor, La Coruña, Spain) and processed as follows. Briefly, sperm-rich ejaculate fractions were obtained, shortly after which they were evaluated for sperm concentration and percentage of motile spermatozoa, diluted in a commercial extender (MR-A®, Las Rozas, Madrid, Spain), then packaged into 100-mL capacity collapsible squeeze bottles (Minitüb, Tiefenbach, Germany) containing at least 3x10⁹ sperm cells per dose. Extended semen not used immediately after collection was kept at 15°C and subsequently used for A.I. within 24 hours.

Oestrus diagnosis was performed once daily at 10:00 a.m. based on the observation of the standing reflex in the presence of a teaser boar combined with manual back pressure. Animals were examined for oestrus every day from day 3 after weaning. Only sows with an average weaning-to-oestrus interval (4-5 days) were included in the experiment. Of these, animals developing short (1 day) or long (3 or more days) oestrus periods were also excluded from the trial. These restrictions were deemed necessary to avoid possible interactions occurring between heat characteristics and the effects of treatment, since the former are known to influence the success of A.I. [23].

Standing sows were inseminated twice: when they were first found in oestrus and again 24 hours later. A management like this (two inseminations per oestrus) is what has been recommended for sows coming normally into heat (4-5 days after weaning, instead of within 4 days -sows that come early into heat- or 6-7 days -sows that come late into heat-) [23], and is currently used in the piggery for this type of sows. At the time of insemination, a breeding belt equipped with a bottle holder (Minitüb, Tiefenbach, Germany) was attached to each sow to allow a hands-free insemination technique. This system appears to save labour because the inseminate penetrates the uterus assisted solely by myometrial contractions, without the bottle having to be held and squeezed, thus making it possible for a number of artificial matings to be simultaneously made upon neighbouring individually penned sows in relatively few minutes. After setting the catheter, the semen bottles were connected and their bottoms pierced with a sharp-ended 18-gauge needle to facilitate the uptake of semen. Only one trained technician performed the inseminations, which took place 45-90 minutes after oestrus diagnosis and without a boar being present.

Experimental Design

A compound seminal additive of O.E. and O.T. [LECHON-PLUS® (L-P) : Oxytocin : 5x10³ IU/L, Estradiol benzoate : 10 mg/L], commercially available in Spain (Porcicón, S.L., León, Spain), was used to study the combined effect of these drugs on semen absorption rate. Two groups of sows were randomly made with no particular criterion for sorting except parity (attention to the weaning-to-oestrus interval remained, as stated previously), since it has been reported that a sow’s age might affect the efficiency of semen deposition [19]. Animals used for this study were between their first and ninth parity, but we attempted to maintain a homogeneous parity distribution in the two groups (Table I). One group of sows received 1 mL of L-P (O.T. : 5 IU ; O.E. : 10 g) added to the semen immediately before use (L-P group), whereas sows in the other group remained as controls and were inseminated with non-treated semen doses. The two inseminations of a sow had the same treatment.

Parameters Evaluated

The duration of semen uptake was measured with digital chronometers, which were started immediately after the bottles were pierced and stopped once they had completely emptied. The time in minutes for the first (A.I.₁) and second (A.I.₂) artificial inseminations was recorded, and the total time of semen uptake (A.I.₁₂) was calculated. When the production data were available [conception rate, as determined by the 21-23 days after insemination non-return-to-oestr-
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trus rate, and prolificacy, as expressed by litter size], then the reproductive performance of each sow in each group was evaluated as litter size (total number of piglets born per litter) per minute of labour (referred to A.I.1+2).

STATISTICAL ANALYSIS

Differences between groups in the average time of semen uptake for single (A.I. 1, A.I.2) and double (A.I. 1+2) inseminations, as well as in litter size and reproductive performance, were compared by using Student’s t-test. Student’s t-test for dependent samples (paired t-test) was used for intra-group comparisons of semen absorption time (A.I.1 vs. A.I.2). Conception rate was analyzed by means of the Yates’ corrected Chi-square test. All statistical analyses were made according to CARRASCO [1] and using the STATISTICA® software for Windows (Release 4.5, Statsoft, Inc, 1993).

Results

Only 43 valuable cases of sows could be included in the L-P group and 41 in the control one. The final parity distribution in these two groups have been shown in Table I. Two sows in the L-P group could not be checked for pregnancy (sale, death) and were consequently excluded from the analysis of conception rate. Sows diagnosed pregnant but having an abortion or a late return to oestrus (four in each group) did not farrow and were excluded from the analysis of reproductive performance.

Tables II and III show the results for all the parameters studied. Use of additive-enriched semen doses only reduced significantly the time required to perform the first insemination (P = 0.025). Total semen absorption time per sow was about 0.70 minutes less in the group receiving L-P, but this difference fell far from being significant (P = 0.14). Nevertheless, a distribution of cases of overall semen absorption time from <1 to >8 minutes appearing in each group distributed over several classes.
absorption time periods in each group is shown in Figure 1, and the fact that virtually all the sows (41 out of 43; 95.35%) inseminated with L-P required less than 6 minutes for this activity to be completed, instead of the lower percentage found in the control group (75.61%; 31 out of 41 sows; \(P = 0.02\) with regard to the L-P group; Yates’ corrected Chi-square test), is noteworthy. For sows receiving doses with L-P, the intra-group difference was also significant, A.I.2 taking more than half a minute longer than A.I.1 (\(P = 0.0011\)). As for production data, neither conception rate nor litter size was significantly improved by using the additive. Reproductive performance between groups, however, did differ significantly (\(P = 0.033\)), since a higher number of piglets per litter per minute of labour was obtained.

Discussion

In our work, we measured the time for semen uptake in terms of labour requirements associated with A.I. This would seem to be of use in making meaningful comparisons between different methods of inseminating, since the other stages of the on-farm A.I. technique (detection of oestrus, semen collection and processing, and equipment cleaning) [7] will not vary greatly from one procedure to another unless sample sizes are substantially different. Therefore, while different insemination techniques do not widely differ in regard to labour demands for the latter tasks mentioned, for practical purposes, time differences in semen deposition may be observed. Our results show that artificially-enriched semen with O.E. and O.T. only induces a faster absorption of semen into the uterine lumen for inseminations performed on the first day of oestrus, at least when inseminating sows in an oestrus stage as described in this paper. Therefore, the interest in carrying out this procedure would be limited, at first, to the inseminations practiced on the first day of oestrus.

The average duration of a single insemination in sows not receiving additive was 2.21 ± 1.43 minutes (A.I.1 and A.I.2 combined). Higher values have been reported by other authors (3.7 ± 0.2 minutes [24]; 4-8 minutes [10]). Variability in experimental conditions, age and oestrus characteristics of sows, existing among these three studies, may account for these differences. The most interesting comparative aspect of our work, however, was the reduction in semen uptake time achieved on the first day of inseminations through hormonal supplementation of semen, since a similar profitability could not be obtained in any of the two previously reported studies using a comparable strategy (exposure to boar [24]; addition of oxytocin [10] and A.I. timing (two inseminations per oestrus, starting the first day of oestrus). Variability in experimental procedures may be behind these differences again. However, the mechanism(s) through which a faster absorption of semen has been induced in our study needs to be elucidated. It may have been effected by increasing the contractile activity of the myometrium, as stated in the introduction. In pigs, it has been shown that intrauterine infusions of O.E. (10 \(\mu\)g in 100 ml of a saline solution) on the first day of standing heat lead to an increase in myometrial contraction frequency due to the stimulating effect of such substances on PGF2\(_{2\alpha}\) synthesis by the endometrium [2]. Oxytocin, for its part, directly stimulates uterine contractility (the so-called “oxytocic effect”) [9]. However, measures of possible differences in myometrial contractility were not taken in our study, and whether the mechanisms of these hormonal effects have occurred under the conditions of our experiment is not known. For instance, the oestrogen effect has been reported to occur within 1 minute after infusion [2], but for other authors [13] it does not become apparent until 10 minutes after the start of infusion. None of those alternatives would explain an influence on semen absorption time with the use of our hands-free approach. Information on how quickly O.T. in semen can increase myometrial contractility is sparse, but it is known that changes in uterine activity can be immediately induced by exposing a sow in oestrus to a boar, because an increase in basal levels of O.T. occurs within 1 minute after boar presentation (returning to basal levels 20-30 minutes later) [14]. Nonetheless, it is clear in our study that the effects on semen uptake must be related to the enrichment of the semen, as the two groups of sows were subjected to the same experimental management. Further research on the mechanism(s) of this induced faster absorption of semen is therefore needed. On the other hand, assuming that an immediate effect on uterine contractility actually occurs, the fact that the procedure was clearly inefficient when practiced 24 hours after the first day of standing heat may be due to the variations in the amount of myometrial oestrogens and oxytocin receptors during the oestrous cycle, as they are known to be at their maximum during late pro-oestrus and decrease progressively thereafter [17, 21]. CLAUS [2] reported the lack of an oestrogen-induced PGF2\(_{2\alpha}\) release when infusing oestrogens on the second day of standing heat, and more recently WILLENBURG et al. [25] found that hormonal supplementation with O.E. or O.T. (but not PGF2\(_{2\alpha}\)) failed to increase the frequency of myometrial contractions in sows inseminated 24 h after the onset of oestrus. However, no explanations for these observations were given.

The beneficial effect of using O.E. and O.T. in A.I. to improve conception rate and litter size is based upon the observations that these substances enhance some aspects of the breeding process (sperm transport and ovulation), but the method has not always been effective, the reason for this is poorly understood [8]. In our study, we did not find any positive influence either on conception rate or on litter size by adding L-P to the insemination doses. However, when reproductive performances are evaluated on a per time of labour basis, significant differences with respect to the control group were actually seen. This means that despite the lesser efficiency of L-P in bringing about a rapid semen uptake on the second day of oestrus, the effects achieved on A.I.1 labour demands appear to be enough for improving the reproductive performance in terms of piglets produced per time of labour.

The deposition of semen appears to be a stage of the pig insemination technique with a highly variable duration among individuals, as it is borne out from the values of standard deviations obtained in our experiment. The strategy of adding myometrial contractions-stimulating substances does

not prevent this event from occurring, yet it proved effective in the first A.I. for saving labour activity time (nearly three quarters of a minute per sow) in such a scenario. However, because of the sole significant effects on A.I. labour demands, only a partial labour saving advantage exists in the technique, with no real maximization of manpower. This finding appears to decrease the interest in performing such a procedure. Large herds require intense artificial mating, therefore a partial advantage like that might not be sufficient to offset the costs of using seminal additives of this type. The expense associated with its use would be balanced with a complete saving in labour costs or comparable high fertility and prolificacy, but for our study the benefits only seem to be related to the concept that manpower could be more productive. A production rate of forty-five more piglets per every hour was achieved with the use of additive-enriched semen doses.

In conclusion, this study predicts the expectation of a reduced labour requirement in inseminations performed on the first day of oestrus by actually adding O.E. and O.T. to the inseminate, along with the attainment of better prolificacy results on a per time of labour basis. The bulk of published work on using these substances has mainly focused on the possibility of improving sows’ reproductive performance. To the authors’ knowledge, this is the first report to the effect that other benefits might also exist. Further large-scale studies would obviously be desirable with the sole addition of oestrogens, oxytocin, or even PGF2α, to test the feasibility of a similar profitability. Sows with different weaning-to-oestrus intervals should also be tested to better understand whether the data of this work might apply to the whole female population in a farm.

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