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Improving of pig production by intrauterine artificial insemination and other biotechnological solutions

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Abstract. Thanks to constantly increasing human population number during the last few decades, the need for pork meat on market increased dramatically. That requirement made imperative for improvement of swine production efficiency. To achieve this goal, it is necessary to implement new biotechnological procedures and production protocols. The use of artificial insemination (AI) in intensive swine production increased notably during the last 30 years and there is a constant need for improvement of AI technologies. New insemination methods (insemination with frozen-thawed semen, different insemination techniques) as well as modern equipment for analysis (CASA microscope, flow cytometry) and sperms' triage (semen sexing), contributed to obtaining much better production results in modern pig industry. The aim of our research was to evaluate sows' reproductive performances after the usage of intrauterine (IU) insemination with reduced semen volume doses and reduced sperm number, in comparison on classical, intracervical artificial insemination. The experiment included a total of 200 sows from 3 pig farms, which were divided in two groups. Test group (n=100) was inseminated applying intra-uterine (post-cervical) insemination technique with special „baloon"catheters, while control group (n=100) was inseminated on the usual manner, using intracervical (IC) technique. Insemination in both groups was done after oestrus detection (standing reflex) in the presence of mature boar. For IU artificial insemination had been used chilled semen doses with 1,5 billion of spermatozoain total volume of 50 ml, while for IC insemination of control group had been used standard semen dose of 100 ml and 3 billion spermatozoa. In both groups, insemination had been done once. Insemination with catheter for IU artificial insemination was successful in 89 sows. From the total number of successfully inseminated sows, 84 sows farrowed (94.38 %), while farrowing rate in control group was 78%. Based on results from this experiment, we determined statistical significant ($p<0,01$) higher number of liveborn piglets after intrauterine insemination (11,00 ± 1,57) with regard to intracervical insemination (10,14 ± 1,10). Applying of intrauterine (postcervical) insemination technique increases a number of spermatozoa that get into oviduct, on site of fertilization. That raises farrowing rate, litter size and number of live born piglets in inseminated sows. In addition, the use of IU catheters decreases semen backflow which allows usage of semen doses of less volume with reduced number of sperm cells. Insemination with this type of IU catheters can't be done if sows aren't in optimal oestrus period. Insemination of double more sows with the same volume of quality semen, better using of genetic material from excellent boars, better assessment of optimal insemination time especially during warm period of the year, are reason good enough for applying IU techniques on pig.

Key words: sows, intra-uterine insemination, biotechnology, reproduction

INTRODUCTION

Artificial insemination(AI) have a very important role in modern swine industry. Beside as a measure of better utilization of production potential, AI is today a part of intensive pig' production routine and it is also a biosafety measure because it stops transmission of infectious and parasitic diseases through semen or by direct contact between animals. During IC insemination of sows, semen dose is deposited in caudal parts of cervix (average 15 cm in

the depth of cervical canal) with the help of a various types of catheters. In practice, the most commonly used insemination doses contains approximately $3{,}25\times10^9$ spermatozoa in 90 ml of extender. How is mainly practiced double insemination of sows, it means that for any successful insemination it needs an average of 6.5×10^9 spermatozoa. Since we know that an average boar ejaculate consist about 70×10^9 spermatozoa, it means that with one ejaculate, 10 to 11 sows can be inseminated (with double insemination). On a monthly basis, from one boar can be taken approximately 5 ejaculates, which is a total of 1300 doses per year (Singleton, 2001). In intensive swine production, this semen production is considered as insufficient, in zootechnical and economic terms (Stančić et al. 2007). By reducing the number of sperm per dose to obtain a higher number of produced doses by a boar, we would have a significant economic savings (Lewis et al. 2002) because it could then be inseminated more females with the same amount of ejaculate (Belstra, 2002; Rozeboom et al. 2004).

When performing artificial insemination with semen doses that have reduced spermatozoa number, we need to be careful not to reduce fecundity and sows' fertility, regarding to keep it up on level of about 85% (Rath, 2002; Stančić, 2002 i Stančić et al. 2003). For this purpose it have been developed numerous new techniques and strategies for artificial insemination, like postcervical (or intrauterine, IU) insemination, when semen is deposited on the beginning of uterus body (Gil et al., 2000, 2004; Watson and Behan, 2002; Roberts and Bilkei, 2005) and deep intrauterine insemination (DIU), when semen is deposited in uterus' horns using non-surgical procedures (Krueger and Rath, 2000; Martínez et al., 2001, 2002).

This techniques are based on primary idea to deposit semen as close as possible to the fertilization site (oviduct). Before meeting with oocytes and begin the process of fertilization, spermatozoa are exposed to the influence of different environments through which they travel. When performing AI, boar spermatozoa are deposited in the wrinkled cervix and soon after, they quickly get into the uterus (Rodríguez-Martínez, 2007). Due to myometrium contractions, a small subpopulation of sperm are very quickly (within a minute) transported from the uterus towards the utero-tubal junction where they colonize sperm reservoir. On that place spermatozoa capacitate and do final preparations for their migration to oviduct, a site of egg fertilization. The vast majority of sperm from the genital tract is removed either through phagocytosis by polymorphonuclear neutrophils, either by retrograde transport - semen backflow through the vulva (Steverink et al., 1998 Matthijs et al., 2000, 2003).

Attempts to prevent semen backflow by tamponade of cervix in order to increase spermatozoa number in uterus' body and oviduct, didn't have success (Pursel, 1982). The backflow volume depends on volume of inseminated semen dose and site of its deposition in female genital tract. It means that with usege of smaller volume dose and by applying IU artificial insemination technique,less spermatozoa lossescan be achieved. From economic point of view, use of the intrauterine method can ensure important savings because of less cost for smaller semen dose (Hernández-Caravaca et al., 2012). The aim of our experiment was quantification of differences in farrowing rate and number of live born piglets after single AI with classical IC catheters in comparison with "balloon" catheters for IU insemination, when we used double smaller dose volume with double reduced sperm number.

MATERIAL AND METHODS

Animals and sperm collection. This experiment included a total of 200 sows from 3 farms (T, M and P)which belongs to the same owner. A total capacity of farm P is 1200 breeding sows and gilts, while farms T and M have 700 animals per each. All farms are built on the same manner and have unique system for food production for all categories of animals. Sows (Landrace X Large White) were different parity (from 3. to 5.). They were housed in

individual pens and fed twice per day and boars were in separate building, in individual pens, also, and fed once per day with addition of vitamin supplement. All animals had ad libidum access to water. From each boar semen was collected once per week with gloved-hand technique. After separation of gel-fraction, macroscopic and microscopic examination of each ejaculate had been done. For acceptation and further processing of ejaculate, it had to meet some requirements: volume – > 120 ml; color – milky white; smell – species specific; number of spermatozoa per ml - 250 x 10^6; agglutination - < 30 % and pH – 7.4 to 7.8. Volume (without gel-fraction) was measured with a direct reading from graduated scale of glass cylinder. Sperm concentration/ml was determined with haemocytometer (Thoma chamber).

Percentage of motile spermatozoa (from 0 to 100%) was determined subjectively under light microscope with 100X magnification. For the purpose of experiment was chosen only semen with more of 80% motile sperm cells. Immediately after semen examination, each ejaculate was diluted with Beltsville Thawing solution (BTS, Minitüb, Tiefenbach,Germany) and stored in 100 ml and 50 ml plastic bottles containing 3 billion spermatozoa (used for IC insemination) and 1.5 billion spermatozoa, respectively (used for IU insemination). Number of sperm cells in doses was checked after dilution. Prepared semen doses were cooled on room temperature for a half an hour and after that were kept in fridge on 17˚C and were used in next 24-48 hours.

Oestrus detection and artificial insemination. Multiparous sows used for breeding were weaned 28±3 days after farrowing. Starting from 3rd day after weaning, oestrus detection was performed once per day by experienced workers by allowing sows nose-to-nose contact with mature boars and applying back pressure. The occurrence of oestrus was defined by the standing reflex in front of a teaser boar and reddening and swelling of the vulva. Animals that had weaning-to-oestrus interval 3-5 days were randomly included in IC or IU group. Those one who get into oestrus after fifth day were included only in IC group. Artificial insemination of all selected animals was carried out 24 hours after oestrus detection, only once.

Intracervical (classical) insemination was carried out with disposable rounded, foam-tipped catheter with a cap on the opposite side. After cleaning the vulva, catheter was inserted through the vagina into cervix in order to achieve "cervical lock". Bottle with semen dose was connected to catheter until it was completely emptied into genital tract, due to myometrium contractions. When empty bottle was taken off, cap was placed on and catheter stayed within 2-4 minutes before removing with the goal to reduce semen backflow.

Intrauterine (postcervical) insemination had been done with specially designed "balloon" catheters for this purpose. Although this catheter reminds on classical IC catheter (rounded, foam-tip) it has essential difference because allows semen deposition directly in uterus' body. Insertion of this catheter is on the same manner like IC catheter.After insertion, technician should wait 1-3 minutes (allowing sow to relax and in the same time, catheter provokes additional antiperistaltic myometrium's contractions). After this time had elapsed, technician need to squeeze semen dose very hard, in order to make great hydrostatic pressure inside the catheter and so cause ejection of inner balloon through the cervix.

Under pressure, whole semen dose gets directly inside the uterus. Insemination was successful if inner balloon-membrane was completely, with its whole length (16,5 cm) expulsed out of catheter's body, which we can see after its removing from the sow. If that wasn't a case, whole procedure was done once again and, if it failed again, insemination was marked as unsuccessful and these sows weren't included statistically in experiment. Pregnancy testing was carried out 28-30 days after artificial insemination using mobile ultrasound device "Tringa Linear vet" (Esoate, Italy). The trial took place in warm period of the year (July and August), when are very often problems in reproduction – worse quality of boar semen (less sperm mobility) and much worse conception in sows.

Statistical analysis. In results' analysis were used descriptive statistical parameters. In testing and determination of statistically significant differences between investigated experimental groups, were used two tests: ANOVA, group test, by which had been done determination of significant differences between treatments, all together; and individual, Tukey test by which had been done determination of significant differences between treatments, individually. Differences considered statistically significant were on levels $p<0.05$ and $p<0.01$. Farrowing rate was calculated after all sows farrowed ([farrowed sows ÷ inseminated sows] × 100.0). For calculation of farrowing rate differences between IU and IC group, we used Chi-squared analysis. Statistical analysis of results had been done in statistic packet Prisma Pad 4.00 and MS Excel.

RESULTS AND DISCUSSIONS

Table1. shows results obtained after intracervical insemination of 100 sows from 3 farms (T, M and P). By statistical analysis it was found that farm P had the highest number of total born and live born piglets, 10.90 ± 0.99 and 10.46±1.00, respectively. Comparing number of total born and live born between all three farms, weren't found statistical significant difference ($p>0.05$). The best farrowing rate was on farm M (86.96%), but there weren't statistical significant difference in farrowing rates between all three farms.

Table 1

Effects of intracervical insemination on reproductive perfomances

Farm	Number of sows (n)	Farrowed sows	Farrowing rate (%)	Total born (mean±SE)	Born alive (mean±SE)
T	27	19	70,37	10,47±1,39	9,84±1,34
M	23	20	86,96	10,45±1,40	9,8±0,89
P	50	39	78,00	10,90±0,99	10,46±1,00
Total	100	78	78,00	10,68±1,21	10,14±1,10

Table 2

Effects of intrauterine (postcervical) insemination on reproductive performances

Farm	Number of sows (n)	Farrowed sows	Farrowing rate (%)	Total born (mean±SE)	Born alive (mean±SE)
T	24	22	91,67	11,27±1,58	10,45±1,14
M	21	20	95,24	11,35±1,56	10,35±1,49
P	44	42	95,45	12,45±1,63*	11,60±1,59*
Total	89	84	94,38	11,88±1,68	11,00±1,57

**Symbol indicates differences between means ($p < 0.01$) within column*

Table 2. shows results obtained after intrauterine insemination of sows. From the total of 100 sows, insemination with "balloon" catheter was successful in 89 animals. The rest of 11 animals were not included in statistical analysis. The analysis shows that we had the best results on farm P, including a total born (12,45±1,63), and live born piglets(11,60±1,59).

Results from this farm are significantly higher ($p<0.01$) comparing to results from farms T and M. The highest farrowing rate was also on farm P (95.45%), whereby weren't found significant difference comparing between farms. The highest farrowing rate after IU insemination was on farm P (95.45%), whereby any significant differences with regard to farms T and M weren't found ($p>0.05$). In regard to applied insemination method – intracervical or intrauterine, by statistical significance' analysis of total born piglets, was found that statistically significant ($p<0.01$) higher number of piglets was farrowed after IU insemination (11.88±1.68) than with IC insemination method (10.68±1.21).

When we compared number of live born piglets, we also found statistically significant higher value in IU group (11.00±1.57) in regard to IC group (10.14±1.10). Mean value for farrowing rate after intracervical insemination was 78.00% and 94.38% after intrauterine insemination. Using Chi-squared analysis we found significant difference between this values. Our results shows that intrauterine insemination with double reduced dose volume (50 ml) and spermatozoa number ($1,5x10^9$)resulted in statistically significant higher ($p<0.01$) farrowing rate (94.38%) comparing to intracervical insemination (78%). Also, number of live born piglets per litter was significantly ($p<0.01$) higher after IU with reduced sperm number than in case of IC insemination with standard dose (11.00 vs. 10.14).

Results from our study are in correlation with some other authors. Vanscicle et al. reported farrowing rate of 92.8% and 11.61of total born piglets per litter, after IU insemination with 1.5 billion spermatozoa in 50 ml of extender (Vanscicle et al., 2002). Stančić et al. in their experiment, conducted duringwarm period of a year, were inseminated sows with 2 billion of sperm and they got better reproductive results after usage of IU then IC insemination method, in terms of fertility and number of total born piglets per litter (75.0% vs. 53.3% and 10.95 vs. 10.12) (Stančić et al, 2013). Numerous researches have been working on problems related to possibilities for usage of reduced semen doses in intrauterine and intracervical insemination technique, parallel. Up to now, one of the biggest studies, conducted on 3240 sows, have been done by Watson and Behan. They used doses with 1, 2 and 3 billion spermatozoa and concluded that reducing of doses up to 1 billion with usage of IU catheters can obtain better results than IC artificial insemination with the same dose (Watson and Behan, 2002.)

In our experiment we got quite worse results after IC insemination, which are in contrast with studies of most the other scientists. This can be explained by the fact that we have been focused on weaning-to-oestrus intervalwhen we choose sows for IU group. Intrauterine insemination has been done only when sows had 3 to 5 days long weaning-to-oestrus interval, because that was the most optimal time for insemination. In contrast to them, sows in IC group have been inseminated during period of 3 to 7 days of weaning-to-oestrus interval. Fallow the management on these farms, all artificial inseminations are performed in two occasions, thus reducing the chances to miss optimal insemination time, which makes theirs results much better (excluding experiment). Since the design of the experiment included one-time insemination, most likely that in some sows insemination was performed too early or too late. In contrary, when using the "balloon" catheter it cannot be the case.

Actually, in 11 sows we couldn't pass the cervix with catheter (because it was closed), so in eleven cases attempts to do IU insemination failed and these sows we didn't included in statistical part of the experiment. At all of the rest sows, catheter was placed very easily, without any resistance, so we could be completely sure that whole semen dose was deposited in uterus. One more very important fact which can influence on insemination succeed is

training of technicians for applying of this procedure. All technicians (two from each farm) were already trained for using of IC catheters. Seven days before experiment started, they had training for work with “balloon” catheters, in order to become professional in this technique. New techniques introduction process can motivate personnel to be, at least temporally, more concentrated on all procedure details, which can improve production results.

The experiment was conducted during summer (Jul and August) when reproductive performances decrease because of high temperatures and longevity of photoperiod. Very common problems in sows are anestrus, late oestrus, decreased farrowing rate and less piglets per litter, as a consequence of implantation and embryo surviving problems (Almond, 1992; Xue et al., 1994). Problems related to boar are reflected as reduced sperm volume and spermatozoa number, with increased percentage of morphological abnormal spermatozoa (Kunavongrit et al, 2005, Chukwuemeka et al., 2005). Our study confirmed that use of IU insemination method during warm period of a year can improve reproductive performances of sows (fertility and fecundity), so negative impacts of high temperatures can be avoided.

CONCLUSIONS

Based on results from our research, we can conclude:

1. With using of intrauterine (postcervical) insemination with double reduced semen dose compared to standard (50 ml, 1.5 billion of spermatozoa) it is possible to achieve much better production results than use of classical intacervical insemination. That is especially important for warm period of a year.
2. Using of reduced semen doses and insemination of double more sows with the same amount of ejaculate, can significantly improve the degree of reproductive exploitation of genetically superior boars
3. Using the "balloon" catheter can easily determine whether is optimal time for insemination, ie. whetherit is cervix open (in that case - there is no resistance when you administer semen doses neither backflow).
4. Intrauterine technique is easy to perform on field conditions and with its usage is possible to achieve the same or even better results than with classic (intracervical) method.
5. However, results of introducing new techniques in intensive production process depends on many different factors (genetic material, farm management, swine diet, technicians who inseminate) so producers should examine efficiency of this methods on their own farms.

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