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# Fertility after deep intra-uterine artificial insemination of concentrated low-volume boar semen doses

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**Abstract**

Boar semen can be successfully frozen – highly packed – in small containers (medium-straw, MS or MiniFlatPack, MFP). The use of deep intra-uterine artificial insemination (DIU-AI) can make possible the deposition of small volumes of this thawed, non re-extended semen deeply intra-uterine, close to the sperm reservoir. The present experiments studied the fertility achieved after single or double DIU-AI per oestrus, with special attention to the interval between AI and spontaneous ovulation. Semen from two boars of proven fertility was frozen in MS or MFP holding $1 \times 10^9$ total spermatozoa. Multiparous (2–5 parity, $n = 42$) crossbred sows were checked for oestrous behaviour after weaning and the occurrence of spontaneous ovulation was checked with transrectal ultrasonography (TUS) to establish the mean interval between onset of oestrus (OO) and ovulation which was found to be when approximately 2/3 of the oestrus period has passed. The sows were, in the following standing oestrus, subjected to DIU-AI using thawed semen from either MS ($n = 20$) or MFP ($n = 22$), inseminated without further re-extension. The sows were randomly allotted to one of three groups: (1) single DIU-AI 8 h before expected ovulation (control group, $n = 19$); (2) single DIU-AI

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4 h before expected ovulation (treatment group S, $n = 15$); and (3) double DIU-AI 12 and 4 h before expected ovulation (treatment group D, $n = 8$). Occurrence of spontaneous ovulation was confirmed by TUS, performed as during the first oestrous period and used to determine the real interval of DIU-AI and ovulation. Pregnancy was also confirmed by TUS 28 days after OO in those sows not returning to oestrus. These sows were slaughtered (30–45 days of pregnancy), and the appearance of the reproductive tract and ovaries, the number of live and dead foetuses, of implantation sites and of corpora lutea (CL) were recorded. Sows ($n = 9$) returning to oestrus (''open'') were re-inseminated (either once [$n = 4$] or twice [$n = 5$]) the following oestrus with either MFP ($n = 5$) or MS ($n = 4$) and slaughtered 12–14 h post-ovulation for recovery of tubal oocytes and of spermatozoa from the uterotubal junctions (sperm reservoir), to assess the degree of effectiveness of sperm transport. Post-thaw sperm motility was 44.3 ± 3.21% in MFP and 42.8 ± 0.72% for MS (LSmean ± S.E.M., n.s.), and did not significantly change from thawing to AI. The DIU-AI could be performed in all sows, but insertion was difficult (slow >5 min) in 5/42 sows. Four of these sows returned to oestrus. Pregnancy rate averaged 35% (group D: 25%, group S: 40%, control: 36%, n.s.). The interval between DIU-AIs and spontaneous ovulation varied largely, ranging from −13 to −3 h for group C, for group S from −11 to +3 h and for group D from −17 to −4 h. Pregnancy rates were clearly related to the interval DIU-AI and ovulation, being highest (60%, 12/20) when AI occurred between 8 and 4 h before spontaneous (not expected) ovulation. The number of implantation sites ranged 6–22 (n.s. among groups), and the number of alive foetuses 2–11 (n.s. among groups). Implantation rate (total number of implantations/CL) ranged 48.0–69.7% being highest in the D-group ($P < 0.05$). The examination of the ''open'' sows slaughtered 12–14 h post-ovulation revealed few recovered oocytes were fertilized (approximately 10%). Only 40% of oocytes had spermatozoa bound to the zona pellucida, not more than two spermatozoa per oocyte. Moreover, low sperm numbers (approximately 4000) were found in the sperm reservoirs (UTJs), irrespective of using single or double DIU-AI (n.s.). The highest values ($P < 0.05$) for these variables were recorded when DIU-AI (either single or double [second AI]) occurred 4–8 h before ovulation, especially when MFP-semen was used ($P < 0.05$). In conclusion: (1) DIU-AI can be easily performed in most sows; (2) pregnancies can be obtained by the DIU-AI of low volumes of highly concentrated frozen–thawed boar semen, once or twice during oestrus, but fertility is still low, probably owing to an unsatisfactory sperm transport when expected and real ovulation differ; and (3) fertility is related to the interval DIU-AI and ovulation which should be −8 to −4 h of spontaneous ovulation and to the package, MFP having shown better results in vivo. The results stress the need for careful, and frequent, control of oestrus signs.


*Keywords:* Frozen semen; Low volume AI; Deep intra-uterine AI; Boar; Pig

## 1. Introduction

Frozen–thawed (FT) boar semen is not regularly used for artificial insemination (AI) in the swine industry; apart from the gene banking of valuable pig genetics, for export or for research purposes. The sub-optimality of the freezing procedure (high cell mortality) and the short-life of the surviving spermatozoa post-thaw accounts, despite the use of large sperm numbers per AI-dose, for the well-documented lower fertility achieved after conventional AI [1,2].

Deposition of reduced sperm numbers through deep intra-uterine AI (DIU-AI) is a relatively new reproductive practice that has drawn the attention of the swine industry. With this technique, the number of frozen–thawed spermatozoa has been substantially