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CURRENT ASPECTS OF ARTIFICIAL INSEMINATION IN PIGS**

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**Plenary invited paper

Introduction

The importance of artificial insemination (AI) in global pig production has increased very strongly. In 2006 about 50% of all sows all over the world were mated by AI. This progress is due to higher concentration of sows per farm. The number of animals on swine farms increased continuously in all pig producing countries. Actually, AI is the most important biotechnical method to realize special aims in breeding and production in all types of swine farm. In 2005 in Germany the share of litters resulting from artificial insemination was about 87% related to total number of litters. In East Germany the share was nearly 100%, in West-Germany about 80% (ZDS, 2006). In fact, the standard boar semen portion for AI contains 2 – 3 x 10^9 sperm. Therefore, it is possible to produce 25 to 30 semen portions per ejaculate (*Rath,* 2001*, Stähr et al.,* 2004). It is thus possible to inseminate 12 to 15 sows twice. With regard to the increased importance of AI in pigs, the demand for semen from boars with top rated genetics has increased very strongly.

There is no longer solely a requirement to reduce the number of sperms in boar semen portions in order to increase the number of portions of semen per ejaculate for AI. The reduction of the sperm content in semen portions facilitates innovation in the reproduction management and breeding systems in pigs. There are three important scientific areas for more effectiveness for AI in pigs:

- Higher utilization capacity rate of boars. It increases the economical and breeding effectiveness of procuring boars with higher genetic potential (*Rath,* 2001*; Geyer,* 2004)
- Possibility to use sex-sorted semen to produce offspring pre-selected by gender. This can be very important for improving reproductive management in piglet production (*Vazquez et al.* 2001*)*.

- Utilization of spermatozoa with reduced fertilization potency after deep freezing

Figure 1 shows the route of the sperm through the uterus and tube through to fertilization. In pigs the ejaculate is deposited into the uterus. Along the route towards fertilization there is a strong selection process. Only a small fraction of inseminated sperm arrives at the location for fertilization. There are some obstacles on the way for the sperm, such as the cervix, and the long distance through the uterus horns in sows, reaching towards 140 cm.

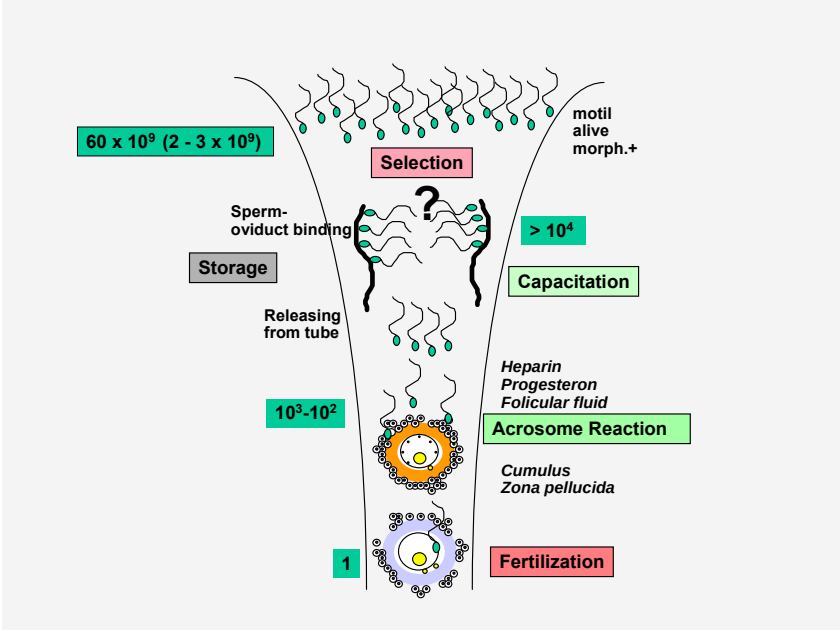

Figure 1. Route of sperm to fertilization (*Müller et al., 2000*)

The transport of spermatozoa in the female reproductive tract to the utero-tubular-combination is a most passive process. Compared with that the further transport to the ampul-isthmic-combination is more active. The influence of seminal plasma and their components like estradiol is very important for the transport of spermatozoa on the way through the uterus and tube (*Claus* 1990). Growing evidence suggests that the flexible oligosaccharide chains of glycoproteins/lipids of the oviductal epithelium "catch" the arriving sperm and hold them back in the sperm storage site of

the oviduct (fig. 2). The corresponding sperm-associated carbohydrate-binding proteins are shed from the sperm surface when the sperm initiate capacitation and hyperactivation (*Töpfer-Petersen,* 2001). A hierarchy of carbohydrate-based interactions exists during the journey of sperm moving towards fertilization. After deposition in the female genital tract sperm are trapped in the isthmic region of the oviduct that forms the oviductal sperm reservoir in sows. Bound to the ciliated epithelium, sperm await the signals announcing the ovulating egg under conditions that maintain sperm viability and suppress the initiation of capacitation. Near the time when the oocyte is ovulated, sperm are released from the oviduct by initiating capacitation, and swim to the site of fertilization to meet the egg.

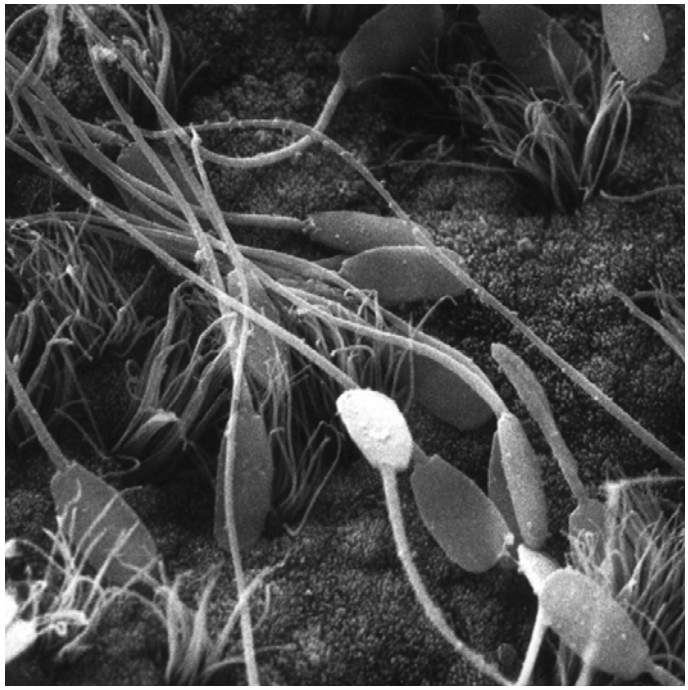

Figure 2. Spermatozoa-oviduct-binding (*Waberski et al. 2004*)

With regard to the selection of sperms on their way to fertilization, it is necessary to deposit semen portions with reduced number of sperms deep intrauterine, much more cranial then in conventional Artificial Insemination with $2x10^9$ sperms per portion (fugure 3).

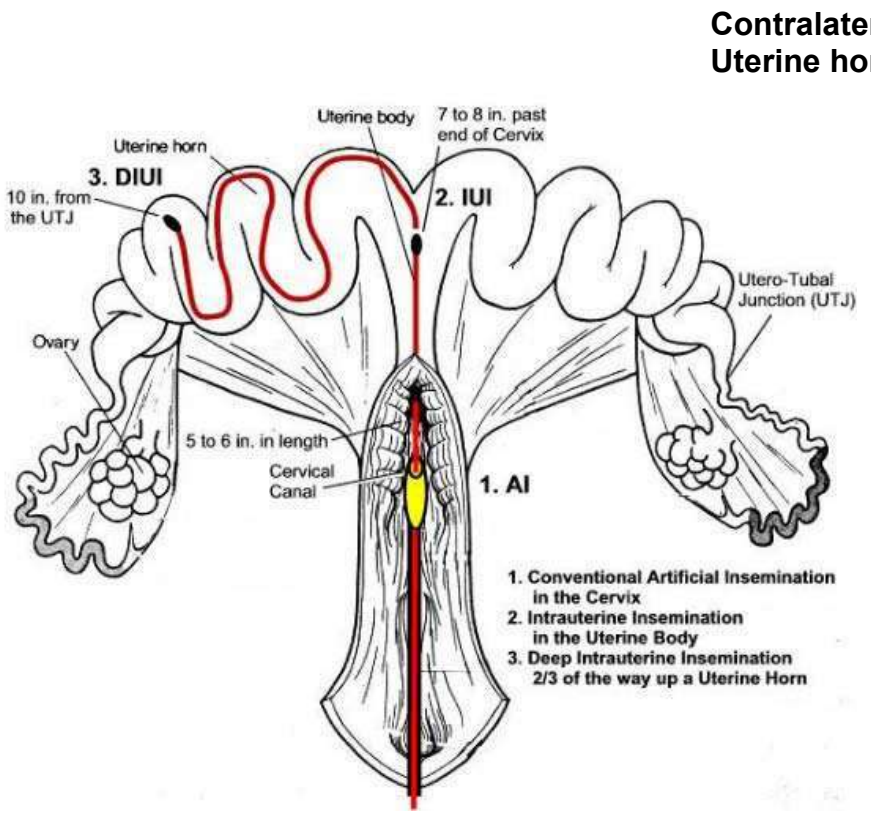

Figure 3. Places for application of sperm in AI

The sperm deposition should be near the place of fertilization, at the end of the uterine horn (*Stähr et al.,* 2004*, Rath et al.* 2003*, Belstra,* 2002). A lot of different results have been published over the past years. The best approach in the application of semen with reduced number of sperms, is the insemination of sows by depositing the spermatozoa at the usual place of fertilization and the first results were obtained using surgical intratubal insemination (*Johnson,* 1991).

However, a surgical procedure is not a practicable technique for insemination for several reasons, including animal welfare issues, costs and time being impractical on-farm. Deep intrauterine insemination is the realistic method for the future. Deep intrauterine deposition of sperm is only possible with a special catheter (Firlflex Magapor, Spain, *Martinez et al.*

2001; fig. 4). Only by this method can a higher level of importance on commercial farms be achieved.

With regard to the first scientific results after surgical and non surgical insemination of sows (*Krüger,* 2000*; Rath,* 2001*; Martinez et al.* 2002) it was necessary to proof the procedure of non surgical deep intrauterine insemination in a commercial swine farm.

Aims of experiment

The goals of the experiment were as follows:

- Test the reproductive performance in sows after deep intrauterine insemination
- Test the catheter-in-catheter system for insemination (Firlflex Magapor) for application on farms
- Calculation of the proportion of sows where the catheter couldn't be used
- Is there an individual effect of boars?
- Is there a risk for internal injuries and damaging affects to the reproductive performance in sows?

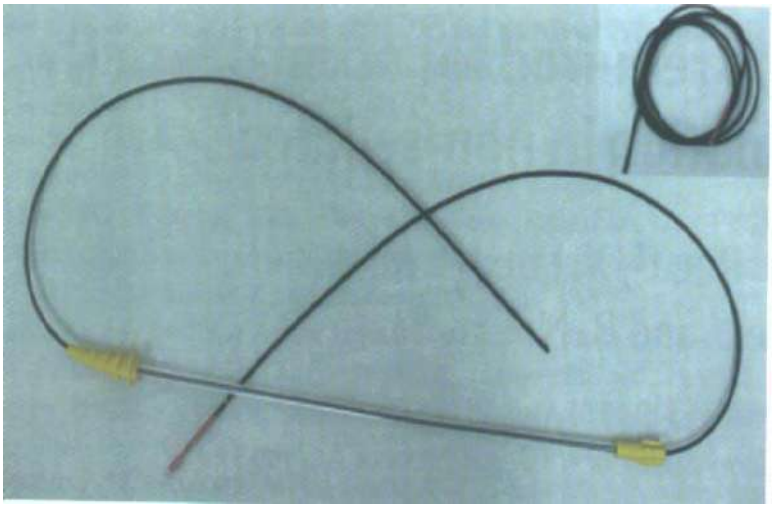

Figure 4. Catheter-in-catheter-System for deep intrauterine insemination, Firlflex, Magapor, Spain, *Martinez et al. 2000*

Material and methods

The experiment was carried out on a commercial farm with in total 2000 sows. Every week, Monday and Tuesday, about 100 sows are ready for artificial insemination, and 90 sows farrowing. 80 sows of Landrace and F1, between Landrace and Large White breeds with 2 or more litters (max. 9 litters) were included in the experiment. They were divided into an experimental and control group. Each group contained 40 sows. After weaning all sows underwent the biotechnical treatment for synchronization of ovulation, which included stimulation of estrus with eCG and stimulation of ovulation with GnRH (*Wähner and Hühn,* 1996*; Wähner* 2002*)*

Procedure for insemination of sows in the control group: Semen portion contained $2x10^9$ sperms in 100 ml seminal fluid. The sows were inseminated twice, 25 and 40 hours after injection of GnRH for stimulation of ovulation. The catheter "Firlflex" Magapor was used for insemination.

Procedure for the insemination of sows in the experimental group: Each semen portion contained 2×10^8 sperms (10% of spermatozoa in a conventional semen portion) in 5 ml seminal fluid. The sows were inseminated once or twice at different times after GnRH. Additionally, the inseminations were carried out at different times after GnRH (table 1).

Table 1. Procedure for deep intrauterine insemination in sows

Replicate	Group	Boar	Interval GnRH – AI (hours)	
			AI 1	AI 2
Replicate 1	Experiment	A	-	40
	Control	A	25	40
Replicate 2	Experiment	A	25	40
	Control	A	25	40
Replicate 3	Experiment	A	25	40
	Control	A	25	40
Replicate 4	Experiment	B, C	30	40
	Control	B, C	25	40

The experiment was repeated 3 times. The boar semen used for insemination came from the Pietrain breed. In replicate 1 to 3 the same boar was used in the control and experimental groups. In replicate 4 two other boars were used. Generally in replicate 1 to 3 the semen of the same boar (boar A) was used for AI 1 and AI 2. In replicate 4 two boars were used in order to find individual effects of the boars for fertilization.

In replicate 1 the sows of the experimental group were inseminated only once. In the subsequent groups they were inseminated twice. The following traits were registered and analysed:

- degree of difficulty for inserting the catheter
- frequency of internal injuries after inserting the catheter
- non-return rate
- pregnancy rate
- litter size

Results and Discussion

The reproductive performance differed considerably between several groups (table 2). Generally, in comparison to animals of the control group, the reproductive performance was reduced. In replicate 1 the results were unsatisfactory. The pregnancy rate was only 44.4% and the litter size 8.25 piglets total born. Therefore, in the next replicates insemination was carried out twice.

Table 2. Reproductive performance in sows after deep intrauterine insemination with reduced number of spermatozoa in semen

	Group	n total/ analysable	Pregnancy NR_{1-28}%	Pregn.-rate %	Litter size: total piglets born x̄	s	Piglets born alive/litter x	s
Trial 1	Experimental group	10/9	50.0	44.4	8.25	1.50	7.75	1.50
	Control group	10/9	90.0	88.8	10.63	3.93	10.00	3.85
Trial 2	Experimental group	10/8	66.6	62.5	8.80	2.95a	8.60	2.88
	Control group	10/10	80.0	80.0	12.63	2.56b	11.38	1.85
Trial 3	Experimental group	10/10	20.0	20.0	8.00	1.00	7.50	0.71a
	Control group	10/8	80.0	75.0	13.33	1.75	13.00	2.00b
Trial 4	Experimental group	10/10	70.9	70.0	10.10	1.20	9.80	0.98
	Control group	10/10	100.0	100.0	11.2	1.98	10.60	1.56
	Experimental group	**40/37**	**51.65**	**49.65**	**8.79**		**8.41**	
	Control group	**40/37**	**87.50**	**85.95**	**11.95**		**11.25**	

The results from all 4 replicates demonstrate a small reduction in reproductive potency in sows after deep intrauterine insemination with reduced number of spermatozoa in the semen. Apart from the fact that in replicate 3, in the experimental group a very low result was realized, the reproductive performance of sows after deep intrauterine insemination was

reduced by nearly 15% for pregnancy rate and by about 25% for litter size. In conclusion, the next experiments should focus on the evenness of the results with more perfect instruments for semen transfer. Furthermore, it is necessary to proof for the possible influence of any pathogens.

The results of the influence of individual boars are very interesting (table 3). After insemination of the semen of boar B all 5 sows in the control group and all 5 sows in experimental group were pregnant. The difference between average litter size in control and experimental group was not significant, the difference was only one piglet. The insemination with semen of boar C in the experimental group produced only 40% pregnancy rate and only 9.00 total born piglets per litter. In the control group the litter size was 10.8.

Table 3. Reproductive performance after different inseminations and dependent on boars

Group / boar	n	Pregnancy rate (%)	Total piglets born per litter		Piglets born alive per litter	
			x	s	x	s
Experimental / B	5	100.0	10.60	1.23	10.20	0.97
Control / B	5	100.0	11.60	1.87	11.20	1.63
Experimental / C	5	40.0	9.00	1.00	9.00	1.00
Control / C	5	100.0	10.80	1.69	10.00	1.46

Table 4. Frequency and degree of difficulties whilst inserting the catheter and internal injuries after inserting the catheter "Firlflex" (Magapor) in sows of the experimental groups (total number of sows)

	AI 1, degree of difficulty*				A 2; degree of difficulty*			
	1	2	3	Blood	1	2	3	Blood
Replicate 1 n = 10	6	4		1				
Replicate 2 n = 10	8	1	1		6	2	1	1
Replicate 3 n = 10	8	2			7	3		
Replicate 4 n = 10	4	6			4	6		

*1…no problems and small problems
2… medium and high degree of problems
3… big problems, catheter was not insertable
Blood…blood was registered on the tip of catheter after insemination

Generally, the frequency of internal injuries in the experimental sows was very low. Only one sow could not be inseminated and in this case the catheter could not be inserted (table 4). Two sows had an internal injury. Blood was registered on the catheter tips. Nevertheless, both sows were pregnant after insemination. But in both cases the total number born and piglets born alive were only 6. In comparison with other experimental sows and control sows, this implies that the litter size was reduced following injuries through insemination with M.

There is a objective risk of transporting pathogens into the deep area of the uterus with the catheter. It can be an additional risk for a reduction in the fertilization rate.

Conclusion

- The catheter-in-catheter-system "Firlflex" Magapor is suitable for deep intrauterine insemination in sows with reduced numbers of spermatozoa in the semen portion. It is partly suitable under commercial farm conditions.
- Generally the frequency of difficulties and internal injuries after insemination is very low. There is only a small risk for a low pregnancy rate. It is necessary to investigate the influence of possible pathogens that are introduced into the uterus via the catheter.
- The deep intrauterine insemination with reduced spermatozoa (10% of conventional dosage) in the pig produces lower reproductive performance. The pregnancy rate is reduced by 15% and the litter size is reduced by 25% in comparison to sows after conventional insemination.
- An individual influence of boars on the reproductive performance after deep intrauterine insemination was registered.
- In case of artificial insemination with "Firlflex" Magapor, only one uterus horn can be used for fertilization. It is not possible to choose the right or left uterine horn.

MODERNI ASPEKTI VEŠTAČKOG OSEMENJAVANJA SVINJA

M. Wähner, M. Geyer

Rezime

Značaj veštačkog osemenjavanja (VO) u globalnoj sinjarskoj proizvodnji je sve veći i veći. U 2006. godini oko 50% svih krmača širom sveta je pareno pomoću VO. Ovakav napredak je uglavnom posledica visoke koncentracije krmača po farmi. Broj životinja na svinjarskim farmama je u stalnom porastu u svim zemljama sa razvijenim svinjarstvom. VO, u stvari, je najvažnija biotehnološka metoda koja se koristi za postizanje određenih ciljeva u odgoju i proizvodnji na svim tipovima svinjarskih farmi. U Nemačkoj, 2005. godine, udeo legala koja su dobijena veštačkim osemenjavanjem je bio oko 87% u odnosu na ukupna broj legala. U istočnoj Nemačkoj ovaj procenat je skoro 100%, u zapadnoj Nemačkoj oko 80% (ZDS, 2006). U stvari, standardna doza semena nerasta koja se koristi za veštačko osemenjavanje sadrži 2 – 3 x 10^9 sperme. Prema tome, moguće je proizvesti 25 do 30 doza semena po ejakulatu (*Rath,* 2001*, Stähr et al.,* 2004). Moguće je osemeniti 12 do 15 krmača dva puta. U vezi sa sve većim značajem VO u svinjarstvu, potražnja za semenom nerastova vrhunske genetike se takođe povećala.

Ciljevi ogleda predstavljenog u radu su bili sledeći:

- Ispitati reproduktivne rezultate krmača nakon duboke unutarmaterične inseminacije
- Ispitati sistem inseminacije kateter-u-kateteru (Firlflex Magapor) odnosno njegovu primenu na farmama
- Izračunavanje udela krmača kod kojih kateter ne može da se koristi
- Da li postoji individualni uticaj nerasta?
- Da li postoji rizik od unutrašnjih povreda i negativnih uticaja na reproduktivne rezultate krmača

Sistem kateter-u-kateter "Firlflex" Magapor je pogodan za duboku unutarmeteričnu inseminaciju krmača sa smanjenim brojem spermatozoida u dozi semena. Delimično je pogodan za primenu u uslovima komercijalnih farmi.

Duboka, unutarmaterična inseminacija krmača sa smanjenim brojem spermatozoida (10% od uobičajene doze) dovodi do lošijih reproduktivnih rezultata. Procenat bremenitosti je smanjen za 15%, a veličina legla za 25% u poređenju sa krmačama koje su osemenjene na konvencionalni način.

Pojedinačni uticaj nerastova na reproduktivne rezultate nakon ovakve inseminacije nije utvrđen.

U slučaju veštačkog osemenjavanja sa "Firlflex" Magapor, može se

koristiti samo jedan rog materice za oplodnju. Nije moguće izabrati desni ili levi rog materice.

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