NON-SURGICAL TRANSFER OF PORCINE EMBRYOS TO DIFFERENT UTERINE SITES AND EFFECT OF TRANSFERRED EMBRYOS NUMBER ON MONITORED PARAMETERS

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Abstract

Embryo transfer in pigs normally involves surgery. In connection with the development of non-surgical transfer techniques, it is important to know whether the uterine site to which embryos are transferred has an effect on the success rate. The aim of this paper was compare two methods of non-surgical embryo transfer. Using the first method embryos were placed into the uterine body, using the second method embryos were placed approximately into the middle of uterine horn. Embryos were recovered post mortem from superovulated donors. Recipients were synchronized by feeding Regumate and by application of PMSG and HCG (gilts) or by piglets weaning (sows). In the first method an insemination probe completed with silicon rigid catheter was used for embryo transfer. Using this probe embryos were put into the corpus uteri. In the second method the intrauterine insemination probe "Verona" completed with silicon rigid catheter was used for embryos transfer. Using this method embryos were put into the middle of one of uterine horns. 42% (10/24) and 56% (12/22) of the recipients remained pregnant after transfer of embryos to the uterus body and to the middle of one of uterine horn. Litter size per pregnant recipients was 5.5 vs. 6.6 piglets, embryos survival rate was 10 vs. 11% (first method vs. second method). Non significant differences were found out between methods.

The second objective of this study was to evaluate the effect of number of transferred embryos on number of born piglets. Recipients were divided into 3 groups according to amount of transferred embryos. From 5 to 15 embryos were transferred into recipients in the first group (A), from 16 to 25 embryos were transferred into recipients in the second group (B) and 26 and more embryos were transferred into recipients in the third group (C). 42, 52 and 50% of the recipients remained pregnant after transfer of 5-15, 15-25 and 25and more embryos. Embryos survival rates were 14, 11 and 7%. Analysis of variance was carried out. There were not found significant differences between groups A-B, A-C and B-C.

Key Words: Pig, reproduction, embryos, non-surgical embryo transfer

Porcine embryo transfer could contribute to the controlling mechanismus of animal reproduction, including transgenesis and cloning (Wall RJ., 1996). Polge and Day (1968) reported some of the first efforts in non-surgical embryo transfer in pigs and obtained three viable conceptuses from one gilt at d 17 after transfer. Although the pregnancy rates were low and litter sizes were rather small, live pigs were obtained following non-surgical embryo transfer (Reichenbach et al., 1993; Galvin et al., 1994). It is difficult to collect and transfer pig embryos using non-surgical techniques because of the anatomical complexity of the cervix and uterus. The cervix is a barrier for transferring porcine embryos non-surgically. Thus, pig embryos are generally collected through surgery or after the slaughter of a donor, and transferred surgically into recipients (Polge, 1982). In the 1990s, new techniques to deposit embryos by using a non-surgical procedure were developed. There are a few reports of transcervical transfers of embryos (Polge and Day, 1968; Reichenbach et al., 1993; Galvin et al., 1994; Hazeleger and Kemp, 1994; Li et al., 1996; Wallenhorst et al., 1999, Říha et al., 2003). Even though these authors have shown that it is possible to pass the porcine cervix.

Several studies have directly or indirectly assessed the appropriate number of embryos to transfer into a recipient.

Material and methods

Donors: Pubertal gilts were superovulated by feeding of Regumate for 15 days and treated with PMSG and HCG. Homospermic doses were used for insemination. Embryos were recovered post mortem from superovulated donors.

Recipients: were synchronized by feeding of Regumate and by application of PMSG and HCG (gilts) or by piglets weaning (sows).

Transferred embryos: Fresh and vitrified – thawed embryos stored in complete conditioned medium MEMD completed with 20% foetal calf serum for 0,5 – 2h at 22°C were used for transfer.

Transefer methods: First method - an artificial insemination probe with silicon (adequately rigid) catheter completed with a syringe containing a culture medium (5ml approx.) was used for embryo transfer. This probe construction makes it possible to penetrate through cervix into the uterus body.

Second method - the intrauterine insemination probe "Verona" was used for embryo transfer. This probe consists of artificial insemination (AI) spirette and inner specially designed flexible catheter (length approx. 1 m, outer diameter of 4 mm). This probe was completed with thin silicon catheter ended with a syringe containing a

culture medium for embryos transport. Construction of this probe makes it possible to penetrate into the uterine horns.

Differences in the proportion of gilts remaining pregnant and embryonal survival rates were tested for significance with t-test. Analysis of variance between groups a-b, a-c, b-c was carried out for comparing the effect of transferred embryo number on born piglets number. Statistical program QC Expert was used.

Results and Discussion

The first objective of this study was to compare two transfer methods – placing of embryos into the different uterine sites. Results are shown in Table 1. After transfer of embryos to the uterus body and to the middle of one of uterine horn, 42% (10/24) and 56% (12/22) of the recipients remained pregnant. There were not significant differences between two sites (P>0.05). Litter size per pregnant recipients was 5.5 vs. 6.6 piglets when transferring embryos to the uterus body and to the middle of one of uterus horns. There were no significant differences too (P>0.05). Wallenhorst et al. (1999) in their study found that pregnancy after transfer of embryos to the middle and caudal quarter of one of the uterine hornsis significantly higher (P<0.01) then pregnancy after transfer into the uterine body. Transfer to the uterine body yielded extremely poor results, corresponding with the disappointing results achieved after most non-surgical embryo transfers (Polge and Day, 1968; Reichenbach et al., 1993, Galvin et al., 1994, Hazeleger and Kemp, 1994; Li et al., 1996).

The second objective of this study was to evaluate the effect of number of transferred embryos on number of born piglets. Recipients were divided into 3 groups according to amount of transferred embryos. From 5 to 15 embryos were transferred into recipients in the first group (A), from 16 to 25 embryos were transferred into recipients in the second group (B) and 26 and more embryos were transferred into recipients in the third group (C) (Table 2). 42, 52 and 50% of the recipients remained pregnant after transfer of 5-15, 15-25 and 25and more embryos. Embryos survival rate were 14, 11 and 7%. There were not found significant differences between groups A-B, A-C and B-C. Berthelot et al. (2007) found that best results, in terms of subsequent in vivo embryo survival, were achieved after transferring 20 embryos. They found significant differences (P<0.001) when 20 blastocystes were trasfered compared to 30 (24.7% vs. 15.5%).

Table 1. Pregnancy after non-surgical transfer of porcine embryos to different uterine sites

Variable	First method (uterus body)	Second method (middle of uterine horn)
Number of recipients	24	22
Embryos transferred per recipient	13.3 ± 3.7	23.5±6.5
Recipients pregnant		
Number	10	12
Percent	42 [a]	56 [a]
Number of born piglets per recipients		
Total	5.6 ± 1.5	6.6 ± 2.0
Alive	5.0 ± 0.9	5.8 ± 1.8
Embryonal survival, % [b]	10 [a]	11 [a]

[a] P> 0.05 (t-test)

[b] (Number of total born piglets/ total number of embryos transferred) x 100 (including all recipients)

Table 2. Effect of transferred embryos number on born piglets number

	Number of transferred embryos		
Variable	A (5 – 15)	B (16 – 25)	C (25 and more)
Number of recipients	19	21	6
Transferred embryos	218	429	189
Recipients pregnant			
Number	8	11	3
Percent	42 [a]	52 [a]	50 [a]
Recipients aborted			
Number	3	4	1
Percent	37 [a]	36 [a]	33 [a]
Number of born piglets per recipients			
Total	7.0 ± 1.1	6.7 ± 2.3	6.5 ± 0.5
Alive	5.0 ± 1.0	6.0 ± 1.9	6.0 ± 0
Embryonal survival, % [b]	14	11	7

[a] P>0.05 (analysis of variance, no significant differences between groups a-b, a-c, b-c)
[b] (Number of total born piglets/ total number of embryos transferred) x 100 (including all recipients)

Conclusion

With this non-surgical transfer technology shipping embryos will became practical. To increase the farrowing rate and embryos survival rate after non-surgical embryos transfer, further methodological improvements are necessary.

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