COLLECTION OF *IN VIVO* PRODUCED PORCINE EMBRYOS

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Abstract

The aim of this work was to obtain utmost high-quality and viable embryos for cryopreservation and transfer. Selected pubertal breeding gilts were used as embryo donors. Gilts were synchronized by feeding of Regumate (active agent: Altrenogest, producer: Hoechst, doses recommended by the producer) for 15 days. Then they were treated with PMSG and HCG. Homospermic doses were used for insemination. Embryos were recovered post mortem from donors aged from 5,5 to 6 days. Various stages of embryos were found. However only embryos in perihatching developmental stages suitable for transfer or cryopreservation were classified as usable. For cryopreservation stages of early and expanded blastocystes were preferred. During two years 526 embryos from 72 gilts have been obtained, 373 of them were usable. It means 5,2 usable embryos per gilt. Total recovery rate was 42.1%.

Introduction

Embryo transfer is applied routinely in cattle, sheep and goats reproduction. However transfer and cryopreservation of pig embryos have encountered numerous problems resulting from the specific features of porcine embryos and from the specific features in physiology and morphology of sows reproductive organs. Porcine embryos are highly sensitive to chilling and that's why cryopreservation is very difficult (Pollard and Leibo, 1994). Success has been confiened mainly to the cryopreservation of embryos at the stage of perihatching blastocystes (Fujino at al., 1993; Nagashima et al., 1995; Kobiashi at al., 1998; Dobrinsky at al., 1999). Special attention was given to the negative consequences of cooling and freezing i.e. intracellular lipids damage and destruction of cytoskeleton, although cryoprotectants were used (Jaskowski at al, 1999). Conventional freezing methods will not work for pig embryos. Which are extremely sensitive to slow cooling. Using a rapid cooling process- vitrification- is thought to outpace the damaging effects of slow cooling (Jeziorkowski at al., 2006). Vitrification allows direct transition between liquid and vitreous phase. OPS (Open Pulled Straw) method is characterised by a very high speed of vitrification without direct transition between liquid and vitreous phase. The OPS methode is appropriate for cryopreservation of perihatching developmental stages of porcine embryos too (Berthelot at al., 2000, 2001). Surgical procedures of embryo recovery and embryo transfer (ET) are also the significant limiting factors for commercial utilization of ET in swine. The non- surgical transfer of porcine embryos has encountered very limited success from its early days. The anatomical structure of the cervix and the small orifice of the cervical channel complicate the penetration of a catheter into the uterus lumen. The methods for porcine ET are still in the experimental phase of their development (Riha at al. 2004).
The aim of this work was to obtain utmost high-quality and viable embryos for cryopreservation and transfer. There are given partial results from years 2005 and 2006 in this study.

Material and methods

Pubertal selected breeding gilts (gilts which were displaced from breeding process) were used as embryo donors. Average age was 7 months and average weight about 130 kg.

Oestrus synchronization:
We used Regumate-porcine preparation (active agent: Altrenogest) for inhibition of cycle. Regumate was fed in feeding mixture for 15 days, doses 20mg per animal and day.
Superovulation:
Gilts were medicated with PMSG (Sergon inj. Sicc., ad us. vet., Bioveta Ivanovice in Haná, Czech Republic) preparation dose 750 i.u. 24 hours after last Regumate dose. 76 hours after injection of PMSG gilts were treated with HCG (Pregnyl 1500, Organon N.V., Oss, Holland or Werfachor, Alvetra – Werfft ag, Austria) dose 350 i.u.
Insemination:
The first insemination with homospermic doses was carried out from 12 to 48 hours after treatment of HCG, when gilts showed standing heat. Double reinsemination followed in 6 and 12 hours.
Embryos were picked up post mortem from donors from 5,5 to 6 days after first insemination. Saws were slaughtered, bled , scalded and then the uterus was taken out, uterus horns were cut off, and embryos were flushed out with Dulbecco PBS solution.
Embryos were found out and classified in laboratory.
Embryos viability:
It was verified by cultivation in IVF conditions

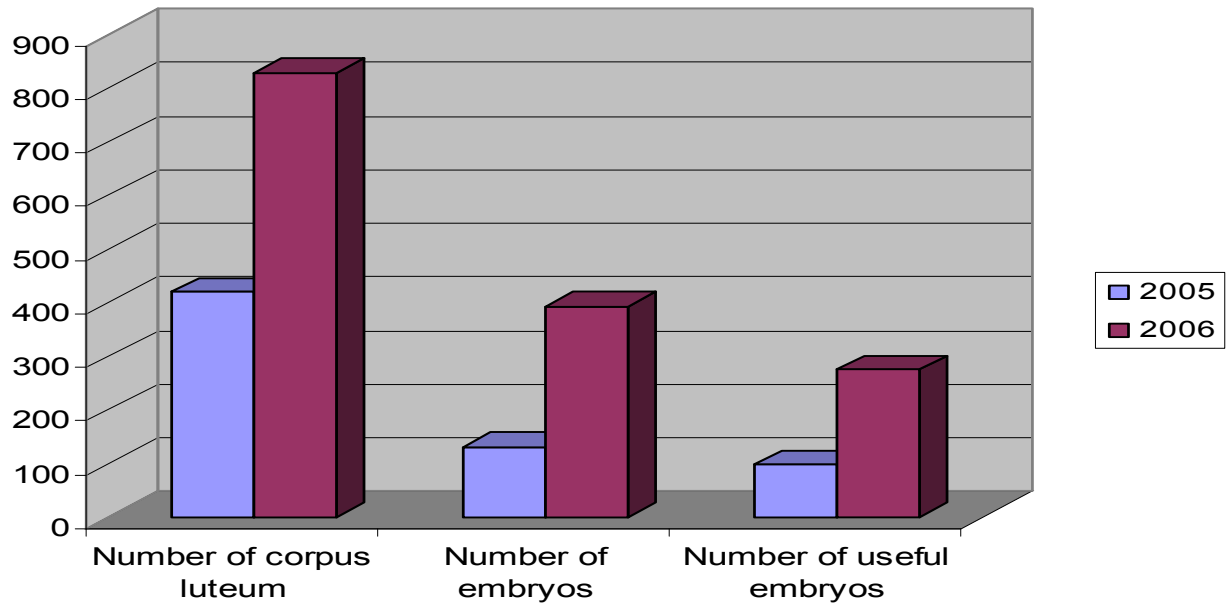
Results and discussion

Number of treated gilts, obtained embryos and another results from year 2005 and 2006 are summarized in Table 1 and Figure 1.

Table 1.: Results from year 2005 and 2006

	2005	2006
Treated gilts	23	49
Number of corpus luteum	420	828
Corpus luteum per gilt	18.3	16.9
Number of follicular cysts	145	88
Follicular cysts per gilt	6.3	1.8
Number of embryos	133	393
Embryos per gilt	5.8	8.0
Recovery rate	31.7%	47.5%
Number of useful embryos	98	275
Useful embryos per gilt	4.3	5.6

Figure 1.: Comparison of selected results in year 2005 and 2006



We found various stages of embryos. Embryos in peri-hatching developmental stages are most suitable for cryopreservation and transfer. Embryos in stages of expanded blastocystes and blastocystes are preferred for cryopreservation and transfer (Říha at al., 2003, 2004). These embryos were classified as usable. Low blastomeres stages of embryos, morules and hatching or after hatching blastocystes are less favoured but usable (Nagashima et al., 1995; Berthelot at al., 2000, 2001). Fragmented and degenerated embryos, unimpregnated oocytes are non acceptable for next using.

There are high differences between results in year 2005 and 2006. These differences could be caused by using different preparation of HCG in year 2005 (Pregnyl 1500) and 2006 (Werfachor). While using preparation Pregnyl 1500 in year 2005 increased incidence of follicular cysts in ovary were observed.

Conclusion

We have obtained 526 embryos from 72 gilts during two years, 373 of them were usable. It means 5,2 usable embryos per gilt. Total recovery rate was 42.1%.

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