Reprod. Nutr. Dev. 40 (2000) 469–480

Original article

In vitro technologies related to pig embryo transfer

Klaus-Peter BRÜSSOW[a]*, Helmut TORNER[a], Wilhelm KANITZ[a], Jozsef RÁTKY[b]

[a] Department of Reproductive Biology, Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany
[b] Department of Reproduction, Research Institute for Animal Breeding and Nutrition, 2053 Herceghalom, Hungary

(Received 2 June 2000; accepted 28 August 2000)

Abstract — Embryo transfer in swine (ETS) has been used for commercial and breeding application only to a limited extent. However this technique is an essential prerequisite for the application of new reproductive techniques in pigs. This paper will give an overview on steps of pig embryo transfer including selection and stimulation of donor sows, recovery of embryos, embryo handling and the transfer of recovered embryos into recipients. Furthermore the current status and further application of ET related in vitro technologies in pig production are described.

pig / embryo transfer / in vitro technologies

Résumé — **Transfert d'embryons chez le porc et techniques associées.** Dans l'espèce porcine, l'utilisation du transfert d'embryons en élevage ou à des fins commerciales est encore limitée. Néanmoins, cette méthode de reproduction par transfert d'embryons est une condition essentielle pour le développement des nouvelles biotechnologies chez cette espèce. Cet article fait le point sur les différentes étapes de la transplantation embryonnaire, y compris la sélection et la superovulation des donneuses, la collecte et la manipulation des embryons. L'état actuel et les applications possibles de la production in vitro d'embryons porcins sont décrits.

porc / transfert d'embryon / fécondation in vitro

1. INTRODUCTION

Global needs for foods and animals require the development of strategies beyond traditional breeding ensuring offspring of value characteristics, of high productivity but maintaining genetic diversity. Oocytes and embryos are escaped from traditional animal breeding methods. However, porcine embryos are an excellent carrier to

* Correspondence and reprints
E-mail: bruessow@fbn-dummerstorf.de

introduce a novel or altered genetic inheritance into individuals and herds. Embryo transfer and associated reproductive techniques enable the manipulation of germ cells and can in conjunction with conventional breeding improve the rate of genetic progress in domestic animal. ETS is a complex of measures including (i) selection and stimulation of donor sows, (ii) recovery of embryos, (iii) embryo handling, i.e. morphological assessment, intermediate storage, cultivation and transport, and (iv) the transfer of recovered embryos into recipients.

In the pig, so far, the application of reproductive techniques is limited. The present paper will focus on the current status of embryo transfer in swine (ETS), on associated techniques of in vitro production of embryos, of sexed and splitted embryos, on cryopreservation of embryos, gentransfer and on its practical application in the pig industry.

Compared to ruminants pig embryo transfer for commercial and breeding applications has been used only to a limited extend. This is due to the high fecundity and to the mainly surgical embryo collection and transfer techniques. First ETS was performed 1951 by Kvasnicki [55] where one out of four recipient sows was pregnant after transfer of 9 embryos, recovered from the oviduct, and 4 piglets were born. Starting from the sixties ETS has been developed and practised [27, 37, 100]. At present embryo transfer in the pig is available as a basic tool of biotechnological procedures.

2. SELECTION OF DONOR SOWS AND SUPEROVULATION

Prepuberal, puberal cycling gilts and sows can successfully be selected as donors. Prepuberal gilts are preferable used because of higher superovulatory response and easy handling. Gilts and sows, especially at the end of their reproductive life, are chosen for the propagation of value breeds.

In pigs, PMSG is exclusively used to induce superovulation (SO). SO is stimulated in prepuberal gilts by application of 1 000–1 500 IU PMSG followed by an injection of 500 IU hCG 72 h after PMSG. In cycling gilts SO is induced after estrus synchronisation (feeding of Regumate® for 15 days) by administration of 1 500 IU PMSG and of 500 IU hCG 78 h later. Multiparous sows are injected with 1 000–1 250 IU PMSG 24 h after weaning and 500 IU hCG 58 h after PMSG. The use of GnRH is an alternative to hCG in triggering ovulation. Gilts and sows are fixed-time inseminated 24 h and 38 h after hCG injection. Results of superovulation and embryo recovery in 146 gilts and sows are presented in Table I.

Gilts and sows show a high variation of ovarian response to superovulatory stimulation. The individual superovulatory reaction,

Table I. Superovulatory response (mean ± SD) in prepuberal and cycling Landrace gilts, and multiparous sows [11, 16].

Parameters	Prepuberal gilts	Cycling gilts	Multiparous sows
Age of animals	180 ± 5	255 ± 5 days	Parity 2–11
No. of animals	83	139	24
No. of ovulation	40.1 ± 12.8^{a}	29.4 ± 12.1^{b}	24.3 ± 7.2^{b}
No. of recovered embroys	32.5 ± 13.6^{a}	23.4 ± 12.8^{b}	19.3 ± 7.4^{b}
No. of transferable embryos	27.2 ± 12.9	20.5 ± 12.8	11.8 ± 8.2
Recovery rate %	81.1 ± 22.1	78.7 ± 24.1	79.0 ± 20.0

[a, b] Means with different superscripts within a row differ significantly ($p < 0.05$).

however, is impossible to predict. Multiparous sows (litter 3–11) demonstrating lasting fecundity (litter size 11.7 ± 2.5) show a less superovulatory response compared to prepuberal gilts.

Likewise, PMSG/hCG preparations (PG 600) [98] or FSH are used to superovulate pigs especially to recover oocytes of preovulatory follicles for in vitro fertilisation [4, 75].

3. EMBRYO COLLECTION TECHNIQUES

No successful non-surgical embryo collection has been reported in pigs so far, except the studies of Kobayashi et al. [50] and Hazeleger et al. [41] following surgical resection of uterine horns. The major reason of this restriction is the anatomy of the porcine genital tract. Therefore embryo collection can be performed in vitro by flushing the genital tract after slaughter, but this technique has the limit to use donor sows only once. Embryo recovery in swine is usually accomplished surgically under general anaesthesia, with the genital tract presented through a midventral incision in the caudal abdominal region involving retrograde flushing of the oviducts and/or uterine horns [10, 24, 36, 97]. The disadvantage of surgical procedures can be diminished by minimal-invasive endoscopic techniques to recover embryos [3, 15, 90].

Embryos can be recovered at different stages of development. Embryos at the one-cell up to the four-cell stage are collected from the oviduct on day 1 to 3 after ovulation, whereas embryos from the four-cell stage up to the hatched blastocyst are flushed from the uterine horn on days 4 to 7 [10].

4. EMBRYO HANDLING

Embryos are morphologically examined in vitro under a stereo microscope after they were isolated from the flushing and transferred into a culture media. Embryos are evaluated according to their morphological characteristics and the expected stage of development referring the day of recovery, and they are classified into transferable or non-transferable embryos. Embryos at the one-cell stage (zygotes) are present in the oviduct on day 1–2. The presence of two polar bodies and of accessory sperm cells is a criterion of fertilisation of one-cell eggs. On days 2–4 embryos are at the two- and four-cell stage within the oviduct. Embryos at the four-cell up to the eight-cell stages can be recovered from the uterine horn between days 4 and 5. Normal developed embryos are in the stage of early to late morula between day 5 and 6, and of early to expanded and hatched blastocysts on days 6–7, respectively. Embryos with a difference of one cell cycle can be accepted as transferable, whereas embryos in difference of more than two cell cycles have to be discharged [11].

Following morphological evaluation embryos are stored for a short time (< 4 h) in a culture medium until they are transferred into recipients. Storage medium has to ensure the viability of embryos during this time. Therefore it is necessary to stabilise temperature, pH, osmolarity and metabolic requirements. Media which not require supplementation of CO_2 during short time culture are useful, like phosphate-buffered saline (PBS) with 3% BSA or HEPES buffered modified Tyrode solution.

5. ET ASSOCIATED IN VITRO TECHNIQUES

5.1. In vitro production of embryos (IVM/IVF/IVC)

5.1.1. Source of oocytes

In the pig 10–20 mature oocytes are ovulated, so that only about 200 oocytes are used for fertilisation during life span.

However, the porcine ovaries contain a relatively large number of primordial follicles (approximately 420 000) [33]. This pool of oocytes is a challenge to use it for in vitro production.

The knowledge of requirements of growing oocytes is still limited [21, 57, 76]. Oocytes derived from preantral follicles grow in vitro up to their final size, acquire meiotic competence and can be penetrated by spermatozoa, but the success rate is still very low. Only 4.8% of oocytes from preantral follicles progress to metaphase II, and 0.4% were penetrated by spermatozoa [42].

The main source of oocytes for in vitro maturation is collected from antral follicles of slaughtered prepuberal pigs. Sorted oocytes with uniform ooplasma and surrounded with compact cumulus may undergo nuclear maturation up to 90% if they were incubated in maturation medium containing porcine follicular fluid and gonadotropins [73]. Usually TCM 199, NCSU 23 or Waymouth Medium were used as basic maturation media.

However, in vitro maturation influences nuclear and cytoplasmatic maturation of porcine oocytes, and subsequent pronuclear formation and first cleavage [56]. Several solutions have been proposed to improve the quality of cytoplasmatic maturation, resulting in higher fertilisability and developmental potential of the in vitro matured oocytes. For example, the gluthathion metabolism seems to be critical in pronuclear formation [2]. The transient inhibition of meiotic resumption has also been shown to improve resulting oocyte quality [30]. Furthermore, the composition of the maturation medium seems determinant of oocyte quality [22].

Difficulties of in vitro maturation are overcome if oocytes are derived from mature prevulatory follicles by means of endoscopic ovum pick up [14]. Cumulus oocyte complexes recovered from PMSG primed pubertal gilts 22–34 h after hCG showed a high incident of cumulus cell expansion (86.7–98.3%) and oocyte nuclear maturation (82.4–100%) [104].

5.1.2. In vitro fertilisation

Pig oocytes matured in vivo and in vitro can be fertilised in vitro using fresh boar semen, frozen-thawed ejaculated sperm or frozen-thawed epididymal spermatozoa [1, 60, 64, 67, 86, 112]. However, differences were obtained between in vivo and in vitro matured oocytes according to sperm penetration rate (69.8 vs. 35.0%) and completion of second meiotic division (41.7 vs. 20.8%). Furthermore the in vitro matured oocytes displayed asynchronous pronucleus development, lower cleavage rate and delayed cleavage [56]. The low incident of male pronuclear formation after in vitro fertilisation is due to suboptimal hormonal conditions, high concentration of NaCl and oxidative stress. However, this problem has been currently solved by modifications of maturation media adding thiols and organic osmolytes [28].

Polyspermy is a lasting problem of IVF. Polyspermic penetration of porcine oocytes range between 13 and 90% [73]. Usually a relatively high number of spermatozoa is added to fertilisation media to maintain capacitation. However a high number of spermatozoa per oocyte is associated with high incident of polyspermic penetration. One possibility to reduce the occurrence of polyspermic penetration is to minimise the number of spermatozoa per oocyte [86]. However a low rate of polyspermic is accompanied by reduced penetration rate [73]. Preincubation with porcine oviductal cells or follicular fluid did not considerably reduce polyspermic penetration [29, 67]. Addition of 10–100 $\mu g \cdot mL^{-1}$ of the porcine oviduct-specific glycoprotein during preincubation and IVF reduced polyspermy rate compared to control (24–29% vs. 61%) [52].

5.1.3. Development of embryos in vitro

Embryos, developed in vivo up to the 8-cell stage, advance under in vitro conditions in a high degree to blastocyst stage. However, the in vitro development from 1- or 2-cell through the 4-cell up to the blastocyst stage is critical. Several media and culture conditions have been created that allow embryo development in vitro [77]. Up to now NCSU23 containing taurin and hypotaurin promote best the development from the 1-cell to blastocyst stage. In NCSU23 90% of in vivo derived and in vivo fertilised 2-cell embryos overcome the 4-cell block and 85% developed to the blastocyst stage compared to 70% and 46% in modified Whittens medium, and 46% and 32% in modified KRB, respectively. However, embryos derived in vivo but fertilised in vitro progress compared to in vivo fertilised embryos a lower rate of overcoming the block stage (57 vs. 92%), and of blastocyst formation (43 vs. 75%) [87]. The cleavage rate of oocytes achieved after in vitro maturation and fertilisation was 73%, and the blastocyst formation rate after 6 day culture was 42%, respectively (Rath, personal information). Although embryos cultured in NCSU23 develop well up to the blastocyst stage they have a lower cell number (28 vs. 59) and embryos are a full cleavage division behind in vivo controls [59]. NCSU23 benefits the embryonic protein metabolism. However, the lower incorporation of methionin into in vitro developed compared to in vivo derived blastocysts demonstrate that cultivation over a longer period has yet to be substantially improved [108].

5.2. In vitro production of sexed embryos

Prediction of sex and production of respective offspring could be a noteworthy application in the pig industry. Sex determination of porcine embryos has been done by chromosomal analysis of cells from blastocysts [47] or by PCR [79]. However these methods are not qualified for practical application in the pig industry to produce sexed embryos. This is due to the high number of embryos necessary for the transfer in the pig, and the lack of effective long-time in vitro culture systems and cryopreservation. Gender preselection by means of high-speed sorting of X- and Y-chromosome-bearing sperm in conjunction with in vitro fertilisation enables the production of sexed embryos and offspring. Both, in vivo and in vitro derived oocytes were fertilised with sexed semen. The cleavage rate was 56.2% and 30.4 to 43.1%, respectively. Altogether 10 and 34 piglets were born, where 100% and 97% were female [88, 89].

5.3. Production of identical multiplets

Embryo bisection, proliferation of single blastomeres, nuclear transfer and parthenogenesis are possible methods to generate identical multiplets. In the pig these methods are rare applied. Piglets were obtained after transfer of bisected blastocysts [69, 92, 94]. Pregnancy rates up to 82% are possible [92], however, the survival rate of demi-embryos is reduced and range between 17% and 34% [12, 69, 92] compared to 52% after transfer of complete control embryos [12]. The outcome of identical twins achieved by this method is low (2.3%) [91].

Single blastomeres derived from 4- and 8-cell embryos developed in vitro, and the blastocyst formation rate ranged between 4–13% [65] and 34–50% [71, 96], but piglets were not produced.

Even though nuclear transfer techniques has been improved in the pig [54, 81, 103], up to now only one piglet was born after transfer of 88 reconstituted embryos which were produced after cloning by fusing single 4-cell blastomeres with enucleated, activated meiotic metaphase II oocytes [80].

5.4. Gene transfer

First transgenic pigs were born 1985 after microinjection of human growth hormone into pronuclei of zygotes [35]. Microinjection of several hundred copies of foreign DNA is still the only promising method to produce transgenic pigs. In the pig, microinjection requires the visualisation of pronuclei by centrifugation. Centrifugation and microinjection influence blastocyst formation. The cleavage and the blastocyst formation rates after 4-days in vivo culture in porcine oviducts were 60% and 38% compared to 74% and 56% of non-injected zygotes, respectively [17]. Besides growth-related genes other gene constructs have been injected into porcine zygotes for different aims (for review see [71, 84]). The efficiency of pronuclear microinjection, however, is still low. About 8% of injected ova result in offspring and 0.7% in transgenic piglets.

Future alternative methods of foreign gene integration into pigs could be sperm-mediated and stem cell-mediated gene transfer. Although after incubation 12–17% of sperm cell were associated with plasmid DNA molecules, none of the piglets born after insemination with DNA-treated sperm showed sign of exogenous DNA incorporation [32]. Destruction of developing male germ cells by busulfan and injection of DNA-liposome complexes transformed spermatocytes. Foreign DNA was expressed in 15–25% of male germ cells [49].

5.5. Cryopreservation

Preservation of embryos over a period of 24–72 h maintaining its viability benefits the collection of embryos for world wide trade. Furthermore, sometimes is a need of holding embryos to collect the optimal number or to find a suitable recipient for transfer. Cryopreservation of porcine embryos would be benefit these requirements. However, routine methods of cryopreservation of porcine embryos are not available. Pig embryos are very sensitive to hypothermic conditions. Although there are reports on birth of live piglets after cryopreservation [38, 46, 51, 66], porcine embryos are limited in their ability to withstand freezing and cryosurvival. This can be overcome using cytoskeletal stabiliser. Embryos cryopreserved by conventional freezing and vitrification under the influence of cytoskeletal stabilisation have resulted in pregnancies (60%) and live offspring after transfer (litter size 5 to 7.3) [25, 26].

6. RECIPIENTS

Selection of recipients has a mayor impact on embryo transfer results. Although prepuberal gilts can be used as recipient, cycling gilts and sows are privileged. This is due to their strong endocrine and uterine condition. The breed of the recipient may be an important factor in pig ET. Meishan gilts, known for their high embryo survival, were successfully used as recipients (pregnancy rate – 86%, 9.3 live born piglets per female) [63].

Recipients are synchronised to donor sows and treated in the same manner except for lower PMSG application (750–1 000 IU) to avoid superovulation. The stimulation of recipients with a lower dose of PMSG creates an endocrine situation which is asynchronous to the donor [13]. An asynchronous transfer (recipients ovulate one day later) benefits embryo survival [78]. The ovarian response of recipients has as well an influence on embryo survival. Recipients with ≥ 6 compared to ≤ 5 ovulation per ovary demonstrated a higher rate of pregnancy (77.8% vs. 55.6%) [8].

Donor gilts have been also used as recipients, reducing the number of animals required for gene transfer programs [6, 84]. However, hormonal secretions after superovulation in donor gilts do not benefit embryo survival and pregnancy [13, 82].

7. EMBRYO TRANSFER

Surgical procedures to transfer embryos into recipients have been available for several decades. The genital tract is exposed under general anaesthesia. The embryos, depended on the stage of development, are transferred either into the oviduct (one- to four-cell embryos) or into the tip of the uterine horn (four-cell embryo to blastocyst). Embryos are transferred in a small amount of medium by means of transfer pipettes or catheters.

Endoscopic procedures were developed recently [3, 91, 102]. This minimal-invasive technique has some advantages compared to surgical procedure but requires endoscopic equipment and experienced handling.

Several attempts were done to transfer embryos non-surgically using AI-spirettes or special designed instruments [31, 39, 58]. Despite several limitations (i.e. use of pluriparous sows only, deposition into the uterine body, stage of embryo development) progress in non-surgical embryo application is promising for expanded application of ETS.

The transfer of 16–20 embryos seems to be optimal to achieve high pregnancy rates [8, 20, 97]. In vitro conditions and manipulation of embryos, i.e microinjection of foreign genes, nuclear transfer, bisection, cryoconservation, limit embryo survival [17, 34, 62]. Therefore, to maximise pregnancy rates a higher number of embryos, that is between 30 and 35, would appear to be optimal for transfer [82, 88, 101, 106].

On average, the pregnancy rate is about 60% and the litter size is 6.5 piglets, with a range of pregnancy from 17% with 2.4 piglets to 100% and litter size of 10.8 piglets [9]. Under optimal conditions, using high quality embryos, pregnancy rates and litter sizes can be obtained similar to results after AI irrespective of the embryo transfer technique, i.e. surgical, endoscopic, non-surgical. Application of in vitro techniques lowers the results of embryo transfer in a wide range. However acceptable results can be achieved observing optimal conditions in all steps. References on results of embryo transfer and related in vitro techniques are shown in Table II.

8. CURRENT STATUS AND APPLICATION

Although embryo transfer techniques have been developed and tested in several fields of swine production, its practical application is still rarely. ETS has been used to introduce new genetic material into closed herds [20, 23, 44] and for extracting healthy stock from diseased source [61, 85, 99]. ET in pigs has been used, but rather to a limited extend, for the export of embryos [45, 72, 109], for the exploitation of superior sows near end of useful reproductive life [16, 48, 61] and for the propagation of endangered swine breeds [91].

Embryo transfer, however, is an essential prerequisite for the application of new reproductive techniques in pigs. Research and initial application in the field of gene manipulation to improve growth and disease resistance, to generate foreign proteins in blood and milk, and to create tissues and organs for xenotransplantation need embryo transfer [7, 18, 19, 35, 71, 95]. Other reproductive techniques to produce offspring like ovum pick up, and in vitro maturation and fertilization [14, 64, 68, 87, 110, 113], generation of sexed embryos [88, 89], cryoconservation of embryos [26, 41, 46] and cloning [12, 69, 80, 94] require also successful handling of embryo transfer technique. However, these reproductive techniques did not find practical application in swine production, yet.

Progress in several steps of porcine embryo transfer like optimized IVC systems, cryopreservation, minimal-invasive recovery and non-surgical embryo application advance further application of this

Table II. Results of embryo transfer following surgical and non-surgical embryo application, and different embryo manipulation.

Application	No. of transfers (*n*)	Pregnancy rate (%)	Litter size (mean)	References
Surgical transfer	27	70	5.7	Dziuk et al. [27]
	77	73	6.2	Schlieper [97]
	46	68	6.7	Kruff [53]
	206	53	7.0	Holtz [43]
	39	80	8.1	Cameron et al. [20]
	112	63	7.7	Brüssow [8]
Mean		68	6.9	
(range)	(27–206)	(53–80)	(5.7–8.1)	
Non-surgical transfer	58	9	5.2	Reichenbach et al. [93]
	21	33	6.7	Hazeleger and Kemp [39]
	46	22	4.3	Galvin et al. [31]
	16	31	6.2	Li et al. [58]
	25	64	3.1	Yonemura et al. [111]
	27	59	10.9	Hazeleger and Kemp [40]
Mean		31	6.1	
(range)	(16–58)	(9–64)	(3.1–10.9)	
Transfer after IVF/IVC	8	38	9	Mattioli et al. [64]
	1	–	3	Yoshida et al. [113]
	3	33	2	Rath [86]
	6	0	0	Rath et al. [87]
	26	35	6.3	Rath et al. [89]
	6	33	2.5	Marchal et al. [60]
Mean		28	3.8	
(range)	(1–26)	(0–38)	(0–12)	
Transfer after gentransfer	10	33	5.0	Brem et al. [5]
	49	59	7.8	Pursel et al. [83]
	98	37	3.9	Springmann et al. [101]
	14	29	4.3	Vize et al. [105]
	39	44	5.9	Brüssow et al. [15]
	17	41	7.1	Williams et al. [107]
	36	81	7.4	Nottle et al. [74]
Mean		46	5.9	
(range)	(10–98)	(29–81)	(3.9–7.8)	
Transfer after bisection	2	–	4	Rorie et al. [94]
	12	42	5.2	Nagashima et al. [70]
	7	14	4	Brüssow and Schwiderski [12]
	27	82	6.0	Reichelt and Niemann [92]
Mean		45	5.6	
(range)	(2–27)	(14–78)	(4–6.0)	

sophisticated biotechnic. One benefit of ETS will be the international exchange of porcine embryos of value breeds instead of live animals. Nevertheless the prospective application of ETS will not become the same significance compared to embryo transfer in other livestock species.

REFERENCES

[1] Abeydeera R.L., Day B.N., Fertilization and subsequent development in vitro of pig oocytes inseminated in a modified tris-buffered medium with frozen-thawed ejaculated spermatozoa, Biol. Reprod. 57 (1997) 729–734.

[2] Abeydeera L.R., Wang W.H., Cantley T.C., Prather R.S., Day B.N., Glutathione content and embryo development after in vitro fertilization of pig oocytes matured in the presence of a thiol compound and various concentrations of cysteine, Zygote 7 (1999) 203–210.

[3] Besenfelder U., Mödl J., Müller M., Brem G., Endoscopic embryo collection and embryo transfer into the oviduct and the uterus of pigs, Theriogenology 47 (1997) 1051–1060.

[4] Bolamba D., Sirard M.-A., Ovulation and follicular growth in gonadotropin-treated gilts followed by in vitro fertilization and development of their oocytes, Theriogenology 53 (2000) 1421–1437.

[5] Brem G., Brenig B., Goodman H.M., Selden R.C., Graf F., Kruff B., Springmann K., Meyer J., Winnacker E.-L., Kräusslich H., Production of transgenic mice, rabbits and pigs by microinjection, Theriogenology 25 (1986) 143.

[6] Brem G., Springmann K., Meier E., Kräusslich H., Brenig B., Müller M., Winnacker E.L., Factors in the success of transgenic pig programs, in: Chruch R.B. (Ed.), Transgenic Models in Medicine and Agriculture, Wiley-Lisys, New York, 1990, pp. 61–72.

[7] Brenig B., Brem G., Genetic engeneering approaches to pig production, Reprod. Domest. Anim. 26 (1991) 14–21.

[8] Brüssow K.-P., Results obtained from transfer of embryos into oviduct and uterus of swine, Mh. Vet. Med. 45 (1990) 562–565.

[9] Brüssow K.-P., König I., Embryotransfer beim Schwein – Stand und Nutzungsperspektiven in der DDR. Tag-Ber, Akad Landwirtsch-Wiss DDR Berlin, 273 (1988) 153–170.

[10] Brüssow K.-P., Kauffold I., Method of embryo transfer in swine, Mh, Vet. Medis 44 (1989) 312–317.

[11] Brüssow K.-P., König, I., Ovarian reaction and embryo quality in donor gilts used for embryo transfer following superovulation stimulation, Mh. Vet. Med. 45 (1990) 143–149.

[12] Brüssow K.-P., Schwiderski H., Results of transfer of porcine split embryos. Arch. Tierz. 33 (1990) 443–447.

[13] Brüssow K.-P., Schneider F., Steroid level in donor gilts following induction of superovulation and its possible influence on embryo quality and transfer results, Mh. Vet. Med. 48 (1993) 405–411.

[14] Brüssow K.-P., Rátky J., Repeated follicular puncture and oocyte aspiration in swine, Reprod. Domest. Anim. 29 (1994) 494–502.

[15] Brüssow K.-P., Rátky J., Endoscopic collection of porcine embryos, Reprod. Domest. Anim. 31 (1996) 711–715.

[16] Brüssow K.-P., Kauffold M., George G., Thieme H.-J., Maaβ P., Use of high-quality adultsows for embryo transfer, Mh. Vet. Med. 44 (1989) 317–320.

[17] Brüssow K.-P., Geissler F., Kauffold M., The influence of microinjection on early embryonic development of porcine zygotes, Arch. Tierz. 33 (1990) 353–356.

[18] Brüssow K.-P., Geissler F., Roschlau K., The influence of microinjection on early embryonic development of porcine zygotes, Arch. Tierz. 33 (1990) 353–356.

[19] Byrne G.W., Mccurry K.R., Martin M.J., Mcclellan S.M., Platt J.L., Logan J.S., Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement-mediated damage, Transplantation 63 (1997) 149–155.

[20] Cameron R.D.A., Durack M., Fogarty R., Putra D.K.H., McVeigh J., Practical experience with commercial embryo transfer in pigs, Aust. Vet. J. 66 (1989) 314–318.

[21] Christmann L., Jung T., Moor R.M., MPF components and meiotic competence in growing pig oocytes, Mol. Reprod. Dev. 38 (1994) 85–90.

[22] Coy P., Ruiz S., Romar R., Campos I., Gadea J., Maturation, fertilization and complete development of porcine oocytes matured under different systems, Theriogenology 51 (1999) 799–812.

[23] Curnock R.M., Day B.N., Dziuk P.J., Embryo transfer in pigs: A method for introducing genetic material into primary specific-pathogen-free herds, Am. J. Vet. Res. 37 (1976) 97–98.

[24] Day B.N., Embryotransfer in swine, Theriogenology 11 (1979) 27–37.

[25] Dobrinsky J.R., Cryopreservation of pig embryos, J. Reprod. Fertil. Suppl. 52 (1997) 301–312.

[26] Dobrinsky J.R., Pursel V.G., Long C.R., Johnson L.A., birth of piglets after transfer of embryos cryopreserved by cytoskeletal stabilization and vitrification, Biol. Reprod. 62 (2000) 564–570.

[27] Dziuk P.J., Polge C., Rowson L.E.A., Intrauterine migration and mixing of embryos in swine following egg transfer, J. Anim. Sci. 23 (1964) 37–42.

[28] Funahashi H, Day B.N., Effect of follicular fluid at fertilization in vitro on sperm penetration in pig oocytes, J. Reprod. Fertil. 99 (1993) 97–103.

[29] Funahashi H., Day B.N., Advances in invitro production of pig embryos, J. Reprod. Fertil. Suppl. 52 (1997) 271–283.

[30] Funahashi H., Cantley T.C., Day B.N., Synchronization of meiosis in porcine oocytes by exposure to dibutryl cyclic adenosine monophosphate improves developmental competence following in vitro fertilization, Biol. Reprod. 57 (1997) 49–53.

[31] Galvin J.M., Killian D.B., Stewart D.K.H., A procedure for successful nonsurgical embryo transfer in swine, Theriogenology 41 (1994) 1279–1289.

[32] Gandolfi F., Terqui M., Modina S., Brevini T.A., Ajmone-Marsan P., Foulon-Gauze F., Courot M., Failure to produce transgenic offspring by intra-tubal insemination of gilts with DNA-treated sperm, Reprod. Fertil. Dev. 8 (1996) 1055–1060.

[33] Gosden R.G., Telfer E., Numbers of follicles and oocytes in mammalian ovaries and their allometric relationships, J. Zool. 211 (1987) 169–175.

[34] Hajdu M.A., Knight J.W., Krisher R.L., Canseco R.S., Velander W.H., Pearson R.E., Gwazdaukas F.C., Effect of culture conditions, donor age, and injection site on in vitro development of DNA microinjected porcine zygotes, J. Anim. Sci. 72 (1994) 1299–1305.

[35] Hammer R.E., Pursel V.G., Rexroad C.E., Wall R.J., Bold D.J., Ebert K.M., Palmiter R.D., Brinster R.L., Production of transgenic rabbits, sheep and pigs by microinjection, Nature 315 (1985) 680–683.

[36] Hammer R.E., Pursel V.G., Rexroad C.E., Wall R.J., Bolt D.J., Palmiter R.D., Brinster R.L., Genetic engineering of mammalian embryos, J. Anim. Sci. 63 (1986) 269–278.

[37] Hancock J.L., Howell G.J.T., Egg transfer in the sow, J. Reprod. Fertil. 2 (1962) 307–331.

[38] Hayashi S., Kobayashi K., Mizuno J., Saitoh K., Hirano S., Birth of piglets from frozen embryos, Vet. Rec. 25 (1989) 43–44.

[39] Hazeleger W., Kemp B., Farrowing rate and litter size after transcervical embryo transfer in sows, Reprod, Domest. Anim. 29 (1994) 481–487.

[40] Hazeleger W., Kemp B., State of art in pig embryo transfer, Theriogenology 51 (1999) 81–90.

[41] Hazeleger W., van der Meulen J., van der Lende T., A method of transcervical embryo collection in the pig, Theriogenology 32 (1989) 727–734.

[42] Hirano Y., Nagai T., Kubo M., Miyake M., Kato S., In vitro growth and maturation of pig oocytes, J. Reprod. Fertil. 100 (1994) 333–339.

[43] Holtz W., Embryotransfer beim Schwein, Tierzüchter 40 (1988) 164–165.

[44] Holtz W., Schlieper B., Stein-Stefani J., Blum B., Agrawala P., Rickert J., Embryo transfer as a means to introduce new stock into SPF pig herds, Theriogenology 27 (1987) 239.

[45] James J.E., Reeser P.D., Davis D.L., Straiton E.C., Talbot A.C., Polge C., Culture and long distance shipment of swine embryos, Theriogenology 14 (1980) 463–469.

[46] Kashiwazaki N., Ohtani S., Nagashima H., Yamakawa H., Cheng W.T.K., Lin A.C., Ma R.C.S., Ogawa S., Production of normal piglets from hatched blastocysts frozen at –196 °C, Theriogenology 35 (1991) 221.

[47] Kato Y., Nagashima H., Shibata K., Ishiwata M., Ogawa S., Microsurgical bisection of expanding blastocysts for the sexing of pig embryos, Jap. J. Anim. Reprod. 33 (1987) 151–154.

[48] Kerr O.M., McCaghey W.J., Ova transfer from aged sows to gilts, Rec. Agricutural Research 32 (1984) 71–76.

[49] Kim J.H., Jung-Ha H.S., Lee H.T., Chung K.S., Development of a positive method for male stem cell-mediated gene transfer in mouse and pig, Mol. Reprod. Dev. 46 (1997) 515–526.

[50] Kobayashi K., Hayashi S., Ohtubo Y., Honda A., Mizuno J., Hirano S., Non-surgical embryo collection from sows with surgically shortened uteri, Theriogenology 32 (1989) 123–129.

[51] Kobayashi S., Takei M., Kano M., Tomita M., Leibo S.P., Piglets produced by transfer of vitrified porcine embryos after stepwise dilution of cryoprotectans, Cryobiology 36 (1998) 20–31.

[52] Kouba A.J., Abeydeera L.R., Alvarez I.M., Day B.N., Buhi W.C., Effects of the porcine oviductspecific glycoprotein on fertilization, polyspermy, and embryonic development in vitro, Biol. Reprod. 63 (2000) 242–250.

[53] Kruff B., Embryotransfer und Bestandssanierung, Tierzüchter 37 (1985) 546–547.

[54] Kühnholzer B., Tao T., Machaty Z., Hawley R.J., Greenstein J.L., Day B.N., Prather R.S., Production of transgenic porcine blastocysts by nuclear transfer, Mol. Reprod. Dev. 56 (2000) 145–148.

[55] Kvasnicki A.V., Novoje v fiziologii rasmnozhenija zhivotnykh, Sel'chosisdat, Moskow 1950.

[56] Laurincik J., Rath D., Niemann H., Differences in pronucleus formation and first cleavage following in vitro fertilization between pig oocytes matured in vivo and in vitro, J. Reprod. Fertil. 102 (1994) 277–284.

[57] Lazzari G., Galli C., Moor R.M., Functional changes in the somatic and germinal compartments during follicle growth in pigs, Anim. Reprod. Sci. 35 (1994) 119–130.

[58] Li J., Reike A., Day B.N., Prather R.S., Technical note: Porcine non-surgical embryo transfer, J. Anim. Sci. 74 (1996) 2263–2268.

[59] Macháty Z., Day B.N., Prather R.S., Development of early porcine embryos in vitro and in vivo, Biol. Reprod. 59 (1998) 451–455.

[60] Marchal R., Feugang J.M., Perreau C., Venturi E.V., Mermillod P., Developmental competence of prepubertal and adult swine oocytes: Bith of piglets from in vitro-produced blastocysts, Theriogenology 53 (2000) 361.

[61] Martin P.A., Commercial embryo transfer in swine: Who is interested and why, Theriogenology 19 (1983) 43–48.

[62] Martin M.J., Houtz J., Adams C., Thomas D., Freeman B., Keirns J., Cottrill F., Effect of pronuclear DNA microinjection on the development of porcine ova in utero, Theriogenology 46 (1996) 695–701.

[63] Martinat-Botte F., Plat M., Procureur R., Despres P., Locatelli A., Berthelot F., Paulmier F., Courot M., Attal J., Houdebine L.M., Terqui M., Use of hyperprolific pig genotypes to improve embryo production and survival in a gene transfer programme, Proc. 6th Scientific Meeting A.E.T.E. Lyon, 1990, p. 174.

[64] Mattioli M., Bacci G., Galeati G., Seren E., Developmental competence of pig oocytes matured and fertilized in vitro, Theriogenology 31 (1989) 1201–1207.

[65] Menino A.R., Wright R.W., Effect of pronase treatment, microdissection and zona pellucida removal on the development of porcine embryos and blastomeres in vitro, Biol. Reprod. 28 (1983) 433–446.

[66] Modl J., Reichenbach H.D., Wolf, E., Brem G., Development of frozen-thawed blastocysts in vitro and in vivo, Vet. Rec. 31 (1996) 208–210.

[67] Nagai T., Moor R.M., Effect of oviduct cells on the incident of polyspermy in pig eggs fertilized in vitro, Mol. Reprod. Dev. 26 (1990) 377–382.

[68] Nagai T., Takahshi T., Masuda H., Shioya Y., Kuwayama M., Fukushima M., Iwasaki S., Hanada A., In vitro fertilization of pig oocytes by frozen boar spermatozoa, J. Reprod. Fertil. 84 (1988) 585–591.

[69] Nagashima H., Kato Y., Shibata K., Production of normal piglets from microsurgically split morulae and blastocysts, Theriogenology 29 (1988) 485–495.

[70] Nagashima H., Kato Y., Ogawa S., Microsurgical bisection of porcine morulae and blastocysts to produce monozygotic twin pregnancies, Gamete Res. 23 (1989) 1–9.

[71] Niemann H., Reichelt B., Manipulating early pig embryos, J. Reprod. Fertil. Suppl. 48 (1993) 75–94.

[72] Niemann H., Wüst A., Gardon J.C., Successful intercontinental transport of porcine embryos from Europe to South America, Theriogenology 31 (1989) 525–530.

[73] Niwa K., Effectiveness of in vitro maturation and in vitro fertilization techniques in pigs, J. Reprod. Fertil. Suppl. 48 (1993) 49–59.

[74] Nottle M.B., Nagashima H., Verma, P.J., Du Z.T., Grupen C.G., Ashman R.J., MacIlfatrick S., Development in transgenic techniques in pigs, J. Reprod. Fertil. Suppl. 52 (1997) 237–244.

[75] Nottle M.B., Verma P.J., Du Z.T., Ashman R.J., Grupen C.J., MacIlfatric S.M., Superovulation of sows with eCG or FSH, Theriogenology 49 (1998) 381.

[76] Petr J., Rozinek J., Fulka J., Jilek F., Influence of cytoplasmatic microinjection on meiotic competence in growing pig oocytes, Reprod. Nutr. Dev. 34 (1994) 81–87.

[77] Petters R.M., Wells K.D., Culture of pig embryos, J. Reprod. Fertil. Suppl. 48 (1993) 61–73.

[78] Polge C., Embryo transplantation and preservation, in: Cole D.J.A., Foxcroft G.R., Control of pig reproduction, London, Butterworth Scientific, 1982, pp. 279–291.

[79] Pomp D., Good B.A., Geisert R.D., Corbin C.J., Conley A.J., Sex identification in mammals with polymerase chain reaction and its use to examine sex effects on diameter of day-10 or -11 pig embryos, J. Anim. Sci. 73 (1995) 1408–1415.

[80] Prather R.S., Sims M.M., First N.L., Nuclear transplantation in early pig embryos, Biol. Reprod. 41 (1989) 414–418.

[81] Prather R.S., Tao T., Machaty Z., Development of the techniques for nuclear transfer in pigs, Theriogenology 51 (1999) 487–498.

[82] Pursel V.G., Wall R.J., Effects of transferred ova per recipient and dual use of donors as recipients on production of transgenic swine, Theriogenology 46 (1996) 201–209.

[83] Pursel V.G., Miller K.F., Pinkert C.A., Development of 1-cell and 2-cell pig ova after microinjection of genes, J. Anim. Sci. Suppl. 65 (1987) 402.

[84] Pursel V.G., Bolt D.J., Miller K.F., Pinkert C.A., Hammer R.E., Palmiter R.D., Brinster R.L., Expression and performance in transgenic pigs, J. Reprod. Fertil. Suppl. 40 (1990) 235–245.

[85] Randall A.E., Pettitt M.J., Plante C., Buckrell B.C., Randall G.C.B., Henderson J.M., Larochelle R., Magar R., Pollard J.W., Elimination of porcine reproductive and respiratory syndrome virus through embryo transfer, Theriogenology 51 (1999) 274.

[86] Rath D., Experiments to improve in vitro fertilization techniques for in vivo-matured porcine oocytes, Theriogenology 37 (1992) 885–896.

[87] Rath D., Niemann H., Torres C.R.L., In vitro development to blastocysts of early porcine embryos produced in vivo or in vitro, Theriogenology 43 (1995) 913–926.

[88] Rath D., Johnson L.A., Dobrinsky J.R., Welch G.R., Niemann H., Production of piglets preselected for sex following in vitro fertilization with X and Y chromosome-bearing spermatozoa sorted by flow cytometry, Theriogenology 47 (1997) 795–800.

[89] Rath D., Long C.R., Dobrinsky J.R., Welch G.R., Schreier L.L., Johnson L.A., In vitro production of sexed embryos for gender preselection: High-speed sorting of X-chromosome-bearing sperm to produce pigs after embryo transfer, J. Anim. Sci. 77 (1999) 3346–3352.

[90] Rátky J., Brüssow K.-P., Successful embryo recovery from swine by minimal invasive technique, Acta Vet. Hung. 43 (1995) 371–375.

[91] Rátky J., Treuer A., Szabo P., Döbrentei B., Soos F., Seregi J., Solti L., Brüssow K.-P., Propagation of endangered swine breed by laparoscopic ET, Theriogenology 47 (1997) 405.

[92] Reichelt B., Niemann H., Generation of identical twin piglets following bisection of embryos at the morula and blastocyst stage, J. Reprod. Fertil. 100 (1994) 163–172.

[93] Reichenbach H.-D., Mödl J., Brem G., Piglets born after transcervical transfer of embryos into recipient gilts, Vet. Rec. 133 (1993) 36–39.

[94] Rorie R.W., Voelkel S.A., McFarland C.W., Southern L.L., Godke R.A., Micromanipulation of day-6 porcine embryos to produce split-embryo piglets, Theriogenology 23 (1985) 225.

[95] Rosengard A.M., Cary N.R., Langford G.A., Tucker A.W., Wallwork J., White D.J., Tissue expression of human complement inhibitor, decay-accelerating factor, in transgenic pigs. A potential approach, Transplantation 59 (1995) 1325–1333.

[96] Saito S., Niehaus P., Niemann H., Developmental ability of bisected and aggregated porcine embryos in vitro, Theriogenology 35 (1991) 267.

[97] Schlieper B., Embryonentransfer beim Schwein – Erfolg in Abhängigkeit vom Induktions- und Gewinnungsmodus, Ph.D. thesis, Göttingen, 1983.

[98] Schlieper B., Holtz W., Some factors affecting the quality of pig embryos collected for transfer, Proc. 10th Internat. Congr. Anim. Reprod. Artif. Insem., Urbana-Champaign, 1984, p. 238.

[99] Singh E.L., Dulac G.C., Hare W.C.D., Eaglesome M.D., Embryo transfer as a means of controlling the transmission of viral infections. V. The in vitro exposure of zona pellucida-intact porcine embryos to African swine fever virus, Theriogenology 22 (1984) 693–700.

[100] Smidt D., Steinbach J., Scheven B., Modified method for the in-vivo recovery of fertilized ova in swine, J. Reprod. Fertil. 10 (1965) 153–156.

[101] Springmann K., Maier E., Binder S., Brem G., Influence of the number of microinjected embryos, transferred, oestrus behavior and body weight of the recipients on the success rate of a gene transfer programme in pig, Theriogenology 29 (1988) 311.

[102] Stein-Stafanie J., Holtz W., Surgical and endoscopic transfer of porcine embryos to different uterine sites, Theriogenology 27 (1987) 278.

[103] Tao T., Machaty Z., Boquest A.C., Day B.N., Prather R.S., Development of porcine embryos reconstructed by microinjection of cultured fetal fibroblast cells into in vitro matured oocytes, Anim. Reprod. Sci. 56 (1999) 133–141.

[104] Torner H., Brüssow K.-P., Alm H., Rátky J., Kanitz W., Morphology of porcine cumulus-oocyte-complexes depends on the stage of preovulatory maturation, Theriogenology 50 (1998) 39–48.

[105] Vize P.D., Michalska A.E., Ashman R., Lloyd B., Stone B.A., Quinn P., Wells J.R.E., Seamark R.F., Introduction of a porcine growth fusion gene into transgenic pigs promotes growth, J. Cell Sci. 90 (1988) 295–300.

[106] Wei Q., Fan J., Chen D., Effect of number of transferred microinjected embryos on pregnancy rate and litter size of pigs, Theriogenology 39 (1993) 338.

[107] Williams B.L., Sparks A.E.T., Canseco R.S., Knight J.W., Verlander W.H., Page R.L., Drohan W.N., Kornegay E.T., Pearson R.E., Wilkins T.D., Gwazdaukas F.C., Evaluation of systems for collection of porcine zygotes for DNA microinjection and transfer, Theriogenology 38 (1992) 501–511.

[108] Wollenhaupt K., Alm H., Brüssow K.-P., Torner H., Characterization of de novo protein synthesis in the early stages of development of porcine embryos collected in vivo and cultured in NCSU-23 medium, Reprod. Domest. Anim. 24 (1995) 133–139.

[109] Wrathall A.E., Done J.T., Stuart P., Mitchell D., Betteridge K.J., Randall G.C.B., Successful intercontinental pig conceptus transfer, Vet. Rec. 67 (1970) 226–228.

[110] Wu G.M., Qin P.C., Tan J.H., Wang L.A., In vitro fertilization of in vitro matured pig oocytes, Theriogenology 37 (1992) 323.

[111] Yonemura I., Fujino Y., Irie S., Miura Y., Transcervical transfer of porcine embryos under practical conditions, J. Reprod. Dev. 42 (1996) 89–94.

[112] Yoshida M., Ishizaki Y., Kawagishi H., Blastocyst formation by pig embryos resulting from in vitro fertilization of oocytes matured in vitro, J. Reprod. Fertil. 88 (1990) 1–8.

[113] Yoshida M., Mizoguchi Y., Ishigaki K., Kojima T., Nagai T., Birth of piglets derived from in vitro fertilization of pig oocytes matured in vitro, Theriogenology 39 (1993) 1303–1311.