In vivo culture of bovine embryos and quality assessment of *in vivo* vs. *in vitro* produced embryos

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ABSTRACT: Routine access to the bovine oviduct for *in vivo* culture accomplishes various demands on embryo production for scientific as well as commercial purposes. The experiments conducted in the present study focused on the efficiency of recovery methods after temporary *in vivo* culture of bovine embryos in oviducts of the homologous species using transvaginal endoscopy (Experiment I) and on the quality assessment of recovered blastocysts (Experiment II). In Experiment I *in vitro* matured oocytes were fertilized, cultured for 1 to 3 days and transferred unilaterally into the ipsilateral oviducts of 54 heifers by the means of transvaginal endoscopy. After 4 to 6 days of *in vivo* culture embryos were re-collected either by non-surgical flushing of uterine horns (U-group) or by combined flushing of the oviducts and uterine horns (OU-group). In total the recovery rate was 38.4% (780/2029). After flushing at day seven, 106 blastocysts (blastocyst rate: 13.6%) were found. The additional 24 h of *in vitro* culture (day eight) resulted in 153 blastocysts (blastocyst rate: 19.6%). The recovery rate in the OU-group was twice as efficient as in the U-group (390/1358 vs. 390/671, $P < 0.01$). The recovery rates among the different stages of transferred embryos did not differ significantly; likewise cross-effects among the stages and the recovery methods were non-significant. The recovery methods ($P < 0.001$) and the interaction between the recovery methods and the stages of transferred embryos ($P < 0.01$) had an influence on blastocyst yields on day seven (U-group 37/1358 vs. OU-group 69/671) and day eight (U-group 48/1358 vs. OU-group 105/671). In Experiment II embryo quality was assessed by the survival rate of blastocysts after freezing in ethylene glycol. Day seven embryos were produced *in vitro* (*in vitro* group D7) or by IVM/IVF followed by a combined culture procedure (2 to 3 days *in vitro* prior to 4 to 5 days *in vivo*) (*in vivo* group D7) or after superovulation and collection at day seven (superovulation group). Embryos from *in vitro* group D7 re-expanded only for 6 h after thawing, embryos from *in vivo* group D7 and superovulation group were alive for 24 h and 72 h of culture, respectively. Only embryos derived by superovulation showed hatching activity. Blastocysts from the *in vitro* group D7 and the *in vivo* group D7 that were held in culture medium for additional 24 h (day eight) showed an analogous post-thawing culture behaviour. In conclusion, the results of the present study demonstrated that some embryos transferred for *in vivo* culture remain in the oviduct even at day seven. Hence, combined flushing of oviducts and uterine horns after *in vivo* culture in the bovine oviduct is necessary for effective embryo re-collection. The quality of recovered embryos after temporary *in vivo* culture assessed by cryotolerance was in-between those produced *in vitro* or recovered after superovulation.

Keywords: cattle; cryotolerance; transvaginal endoscopy; oviduct

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In vitro production (IVP) has become important for the production of bovine embryos as an alternative to or integrated with multiple ovulation and embryo transfer (MOET) due to it´s advantages and flexibility (Galli et al., 2001). Blastocyst yield after IVP is mainly affected by the intrinsic quality of the oocytes, whereas blastocyst quality is determined by the culture environment after fertilization (Rizos et al., 2002). Four important events occur in these seven days of culture period: the first cleavage, the activation of the embryonic genome at the 8- to 16-cell stage, the compaction of the morula on day five and the blastocyst formation accompanied by the formation of the first two embryonic cell lines. Any or all of these events could be affected by inadequate culture conditions and therefore exert negative influences on blastocyst quality (Lonergan et al., 2003b). Hence the quality of *in vitro* produced blastocysts differs not only among the different culture systems but mainly from the quality of embryos produced *in vivo* (Thompson, 1997; Enright et al., 2000; Crosier et al., 2001). These observations were confirmed not only by the assessment of morphological structures, but also by gene expression studies and the postnatal large offspring syndrome (Niemann and Wrenzycki, 2000; Lazzari et al., 2002; Tesfaye et al., 2004).

Up to now *in vitro* conditions cannot mimic the dynamic changes of oviduct and uterus secretion that respond to the varying metabolism of a developing embryo (Bavister, 1995). Therefore only the physiological environment of the preimplantation embryo, e.g. the site of fertilization and early embryo development, provides optimal conditions for embryonic growth up to the blastocyst stage. Successful *in vivo* culture of *in vitro* matured (IVM) and *in vitro* fertilized (IVF) bovine embryos in the bovine, ovine and rabbit oviduct was already proven and pregnancies in recipient animals were established (Boland, 1984; Xu et al., 1987; Galli et al., 2003). In most of the cases rabbit and ovine oviducts were ligated and the recipient animals were used irrespective of the stage of oestrous cycle (Eyestone et al., 1987; Lonergan et al., 2003b). In contrast, the cattle oviduct was not ligated for technical reasons to recover embryos from the uterus on day seven by non-surgical flushing (Xu et al., 1987; Greve et al., 1989). In order to provide the optimal requirements for early embryo-maternal communication it is inevitable to synchronize the oestrous cycle of the recipient and the embryonic stage. The tubal dynamics including the transportation of embryos towards the uterotubal junction are known to be strongly regulated by endocrine and neural mechanisms which lead to a timely entry of the embryo into the uterine horns. The early entry into the uterus may be incompatible with normal subsequent embryonic development (Greve and Callesen, 2001).

The assessment of embryo quality can be primarily evaluated by recording the pregnancy rates or the survival parameters from the post-thawing culture period. *In vitro* produced embryos showed an increased chilling and freezing sensitivity associated with their increased lipid content, a relatively smaller inner cell mass and differences in ultrastructural features compared to *in vivo* produced embryos (Iwasaki et al., 1990; Pollard and Leibo 1994; Abe et al., 1999). *In vivo* culture of bovine embryos in the sheep oviduct improved freezing resistance compared to *in vitro* cultured bovine embryos (Enright et al., 2000; Rizos et al., 2002).

The aim of our study was to examine the efficiency of recovery methods after temporary *in vivo* culture of bovine embryos in oviducts of the homologous species using transvaginal endoscopy. The quality of recovered blastocysts was evaluated by survival after cryopreservation compared to embryos produced *in vitro* or by superovulation.

MATERIAL AND METHODS

In vitro production of embryos

Ovaries collected from a local abattoir were transported to the laboratory in PBS at 25°C to 30°C. Cumulus oocyte complexes (COC) were obtained by aspirating 2 to 8 mm follicles. Only COC enclosed in more than five layers of cumulus cells and with a homogeneous ooplasm were used for *in vitro* maturation. Groups of 25 to 50 oocytes were transferred into 400 µl of maturation medium. IVM was performed in TCM-199. One milliliter TCM-199 was supplemented with 1.4 mg Hepes, 0.8 mg $NaHCO_3$, 0.25 mg pyruvate, 50 µg gentamycin, 4 IU FSH (Folltropin-V®, Vetrepharm, London, Canada) and 10% (v/v) oestrus cow serum (ECS). Oocytes were matured for 24 h in humidified atmosphere composed of 5% CO_2 at 39°C.

After the maturation period oocytes were put into culture wells containing 400 µl of fertilization medium (Fert-TALP supplemented with 6 mg/ml

BSA and 20 µg/ml heparin). Frozen semen from the same bull of known fertility was used for the production of embryos in all groups of the experiments. Frozen-thawed spermatozoa were swim-up-separated in modified Tyrode's medium (Sperm-TALP) as described by Parrish et al. (1986) and added to fertilization medium at a final concentration of 1×10^6 spermatozoa per ml. Oocytes were incubated with spermatozoa for 20 to 22 h in humidified atmosphere and 5% CO_2 at 39°C.

After fertilization the remaining cumulus cells were removed by vortexing. Presumptive zygotes were washed twice in culture medium (amino acid supplemented CR1 medium (Rosenkrans and First, 1994) supplemented with 10% (v/v) oestrous cow serum) and transferred into 400 µl of culture medium covered with mineral oil. Culture was performed at 39°C in humidified atmosphere containing 5% CO_2 and 5% O_2. The number of cleaved embryos was determined after 24 h of culture. One part of the embryos was allocated to the *in vivo* culture experiments, whereas the remaining embryos were kept *in vitro* until day eight. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

Recipient animals

Fifty-four recipient heifers aged between 18 to 20 months weighing 350 to 450 kg were used for *in vivo* culture. Animals were pre-selected based upon normal oestrous activities. The recipient animals were synchronized following an Ovsynch protocol (Mialot et al., 2003). On day five to ten, 0.05 mg GnRH (Supergestran, Biopharm, Jilove, Czech Republic) per animal were injected. Seven days later animals received 500 µg cloprostenol (Oestrophan, Bioveta, Ivanovice na Hane, Czech Republic). Synchronization was finished 48 h later by administration of 0.05 mg GnRH. Ovulation was expected within 26 to 40 hours. This protocol assured the synchronity of recipients with the transferred embryos.

In vivo culture

After one to three days of *in vitro* culture presumptive zygotes or 2-cell to 8-cell stage embryos were aspirated into a glass capillary (Transferpettor Caps®, 100 to 200 µl, No. 701910, Brandt, Wertheim, Germany), which was crosier shaped bent at the tip. The capillary was connected to a flexible tube (perfusor tube, No. 08272514, Braun, Melsungen, Germany) and a 1 ml syringe. Embryos were transferred into the ipsilateral oviducts of synchronized recipients by transvaginal endoscopy as described (Besenfelder and Brem, 1998). Briefly, restrained animals received an epidural anesthesia and the genital region was cleaned and disinfected. An universal metal tube (Storz, Vienna, Austria) bearing a mandrin with a truncated tip was placed middorsal and cranial of the fornix vaginae. The mandrin was replaced by a traumatic trocar and the system was introduced through the abdominal wall into the peritoneal cavity. After removal of the traumatic trocar the tubing system (Storz, Vienna, Austria) containing the endoscope (5.5 mm 0° forward Hopkins endoscope, Storz, Vienna, Austria) and the transfer system was inserted into the universal tube. Visualization was accomplished by a cold light fontain (250 Watt, Storz, Austria) and a camera (Telecam PAL-Endovision, Storz, Vienna, Austria) connected to a monitor. After visualization of the reproductive organs the ovaries were fixed per rectum and quality and side of ovulation were assessed. The glass capillary was inserted via infundibulum 5 to 8 cm deep into the ipsilateral oviduct where embryos were deposited. Before removal of the endoscope the air from the peritoneal cavity was removed using a vacuum pump.

At day seven (*in vitro*: 1 to 3 days; *in vivo*: 6 to 4 days) embryos were re-collected either from uterus by non-surgical flushing (Holy et al., 1990) or from oviduct and uterus by endoscopic flushing of the oviducts and following non-surgical flushing of the uterus (Besenfelder et al., 2001). Briefly, prior to endoscopic insertion, an uterine flushing catheter was positioned near the tip of the uterine horn according to the side of transferred embryos for *in vivo* culture. The access to the peritoneal cavity for endoscopic tubal flushing was as described above. The flushing system consisted of a 20 ml syringe, a perfusor tube and a curved metal capillary (14 cm × 2.5 mm) covered by an elastic balloon. For tubal flushing the capillary was inserted into the ampulla, which showed a flushing pressure dependent increase of diameter during medium flow through the oviduct. The oviducts were flushed with 40 to 60 ml of flushing medium (PBS supplemented with 1% bovine serum) to ensure that embryos passed the uterotubal junction. Finally, the uterine horns were thoroughly rinsed with 300 to 500 ml of flushing

medium by an embryo flushing catheter used for conventional day 7 embryo collection in superovulation programs. The embryos were collected in an embryo filter (Em con filter®, No. 04135, Immuno Systems Inc., Spring Valley, WI).

In all animals the total numbers of recovered oocytes/embryos were calculated as the number of oocytes/embryos reduced by one oocyte originating from the recipient´s ovulation. The blastocyst rates (blastcysts/recovered embryos) and the blastocyst yields (blastocysts/transferred embryos) were recorded on day seven and all stages were additionally incubated in culture medium for 24 h for determination on day eight.

Regarding the time exposure for transfer and recovery, the transfer itself took about 2 to 5 minutes and the re-collection procedure including unilateral flushing of oviducts and uterine horns was performed within a time interval of 20 to 25 minutes.

In vivo production of blastocysts

The protocol used for the *in vivo* production of embryos was as described by Lopatarova et al. (2002). Healthy donor cows were superovulated in the luteal phase (between day 8–12) with eight doses of 480–560 IU of FSH per animal (Folicotropin, Spofa, Prague, Czech Republic) administrated at 12 h intervals. Oestrus was induced by two prostaglandin applications of 500 µg cloprostenol (Oestrophan, Bioveta, Ivanovice na Hane, Czech Republic) at the time of the fifth and the sixth FSH injection. Three artificial inseminations were done at 48, 60 and 72 h after induction of luteolysis. Day seven embryos were recovered by non-surgical flushing of the uterus nine days after prostaglandin administration (Holy et al., 1990).

Embryo freezing

Blastocysts and expanded blastocysts were frozen in ethylene glycol (1.5 mol/l ethylene glycol with 0.1 mol /l sucrose, AB Technology, USA). The embryos were loaded into 0.25 ml straws and placed in a programmable freezer pre-cooled to –7°C. After 5 min seeding was induced. After further 5 min at –7°C straws were cooled at 0.5°C/min to –35°C. Subsequently they were plunged into liquid nitrogen and stored.

For thawing straws were held in air for 10 s, then in a 30°C water bath until the ice in the straws had melted, followed by emptying the contents into culture medium. After three times washing in culture medium the embryos were cultured in groups in 400 µl of culture medium under oil and examined at 6, 24, 48 and 72 h post-thawing. Re-expanded embryos with advanced development were considered viable. In addition, the hatching rate was recorded.

Experimental setups

Experiment I. Experiment I (Exp. I) was performed to find out whether embryos migrate in uterine horns or retain in the oviduct during *in vivo* culture. At day seven embryos were recovered either from uterus by non-surgical flushing (U-group) or from oviduct and uterus by endoscopic flushing of the oviducts following non-surgical flushing of the uterus (OU-group).

Experiment II. The aim of experiment II (Exp. II) was to compare the survival rate of day seven blastocysts after freezing and thawing in ethylene glycol. Embryos were produced (i) *in vitro* (*in vitro* group D7), (ii) by IVM/IVF followed by a combined culture procedure (2 to 3 days *in vitro* prior to 4 to 5 days *in vivo*) (*in vivo* group D7) or (iii) after superovulation and collection at day seven (superovulation group).

Two batches of blastocysts (*in vitro* group D8, *in vivo* group D8) were held in culture medium for additional 24 h before cryopreservation was performed.

Experimental procedures were performed in accordance with Czech legal requirements for animal experimentation.

Statistical analysis

For statistical analysis, we employed a full factorial ANOVA model with method and stage as fixed factors and three different dependent variables: embryo recovery rate, blastocyst yield on day seven and blastocyst yield on day eight. The statistical unit was the recipient cow. Calculations were performed using the *R* statistical package. Blastocyst survival rates after cryopreservation were analysed by chi-square test. A *P*-value less than 0.05 was considered significant.

RESULTS

Experiment I

In all animals, embryo transfer and embryo recollection was performed successfully. In total, 2 029 presumptive zygotes and early cleaved stage IVP embryos were transferred into the ipsilateral oviducts of 54 recipients and 780 embryos (recovery rate: 38.4%) were regained. After flushing at day seven, 106 blastocysts (blastocyst rate: 13.6%) were found. The number of blastocysts increased to 153 after one additional day of *in vitro* culture (blastocyst rate: 19.6%).

The transfer of 1 358 embryos into the oviducts followed by flushing of the uterine horns (U-group) succeeded in 390 re-collected embryos (28.7%), whereas the combined flushing of the oviducts and uterine horns (OU-group) was about twice as efficient (390/671, 58.1%). This difference among methods was significant ($P < 0.01$). No significant differences were observed among the different stages of transferred embryos; likewise cross-effects among the stages and the recovery methods were non-significant (Table 1, Figure 1A).

The blastocyst rates (in relation to the recovered embryos) on day seven and on day eight from the U-group were 9.5% (37/390) and 12.3% (48/390) respectively, whereas the embryos from the OU-group developed to blastocysts at higher rates both on day seven (17.7%, 69/390) and on day eight (26.9%, 105/390). As with the total embryos recovered, the blastocyst yield (in relation to the transferred embryos) both on day seven and on day eight significantly depended on the recovery method ($P < 0.001$). In contrast to the total recovery rate, the cross effects among methods and stages were significant ($P < 0.01$) on both day seven or on day eight. The number of blastocysts recovered at day seven and day eight in the U-group decreased with transfer at a more progressed stage, while in the OU-group it increased (Table 1, Figure 1 B, C).

Neither bleeding nor hyperaemia was observed after manipulation of the oviduct for transfer and re-collection. After *in vivo* culture none of the recipients showed any irregular symptoms in cyclic activity and all animals were re-used for commercial animal breeding.

Experiment II

In total 215 blastocysts were frozen on day seven and 94 blastocysts were cryopreserved on day eight. There was a clear correlation between embryo production and survival rate after thawing: day seven embryos of the *in vitro* group D7 only re-expanded for 6 h after thawing, embryos from

Table 1. *In vivo* culture of different embryonic stages in the bovine oviduct recovered by solely flushing of the uterine horns (U-group) or by combined flushing of the oviducts and the uterine horns (OU-group)

Recovery system	Stage of transferred embryos (day of oestrous cycle and *in vitro* culture)	Embryos transferred, *n*	Embryos recovered, *n* (recovery rate %)	Blastocysts, *n* (blastocyst rate %)	
				Day 7	Day 8
U-group	Zygote to 2-cell stage (Day 1)	675	189	17	24
	2- to 4-cell stage (Day 2)	445	132	18	20
	4- to 8-cell stage (Day 3)	238	69	2	4
	Total	1 358	390 (28.7)	37 (9.5)	48 (12.3)
OU-group	Zygote to 2-cell stage (Day 1)	196	105	5	6
	2- to 4-cell stage (Day 2)	180	108	21	39
	4- to 8-cell stage (Day 3)	295	177	43	60
	Total	671	390 (58.1)	69 (17.7)	105 (26.9)

Recovery rate: recovered/transferred embryos

Blastocyst rate: blastcysts/recovered embryos

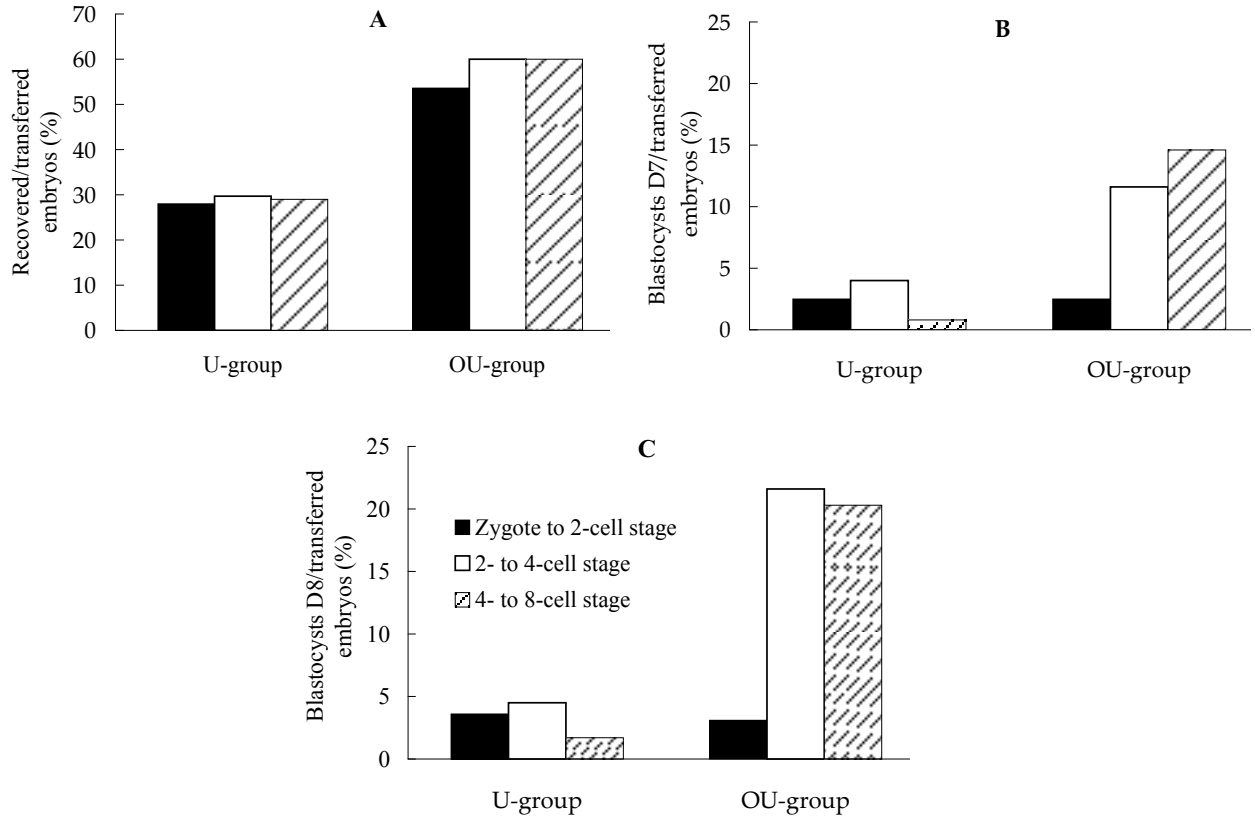

Figure 1. Influence of the recovery method on the collection rate (A) and the blastocyst yield on day seven (B) and on day eight (C) after *in vivo* culture of different embryonic stages in the bovine oviduct. The collection was performed either by solely flushing of the uterus (U-group) or by combined flushing of the oviduct and uterus (OU-group). The recovery rate (A) differed significantly among collection methods ($P < 0.01$). The blastocyst yields both on day seven and on day eight (B, C) significantly depended on the recovery method ($P < 0.001$) and the cross effects among methods and stages ($P < 0.01$)

Table 2. Development of cryopreserved/thawed day seven and day eight bovine blastocysts produced *in vitro* (*in vitro*), by IVM/IVF followed by a combined culture procedure (2 to 3 days *in vitro* prior to 4 to 5 days *in vivo*) (*in vivo*) or after superovulation and collection at day seven (superovulation)

Production of embryos	Number of embryos (*n*)	Post-thawing development, *n* (%)				
		6 h	24 h	48 h	72 h	Hatched
Day 7						
Superovulation	84	81 (96.4)^{a}	77 (91.7)^{a}	45 (53.6)^{a}	29 (34.5)^{a}	29 (100)^{a}
In vitro	75	30 (40.0)^{b}	0^{c}	0^{b}	0^{b}	0^{b}
In vivo	56	50 (89.3)^{a}	8 (14.3)^{b}	0^{b}	0^{b}	0^{b}
Day 8						
In vitro	40	18 (45.0)^{a}	0^{a}	0	0	0
In vivo	54	45 (83.3)^{b}	8 (14.8)^{b}	0	0	0

The percentage of hatched embryos was based on 72 h surviving embryos
Within day seven and day eight and in the same columns, values with different superscripts a, b, c are significantly different ($P < 0.05$)

the *in vivo* group D7 and the superovulation group were alive for 24 h and 72 h of culture, respectively. Only embryos derived by superovulation showed hatching activity. The two groups of day eight embryos showed an analogous post-thawing culture behaviour (Table 2).

DISCUSSION

The present study aimed at optimising the embryo recovery system following *in vivo* culture and at evaluating three different embryo culture systems by assessment of embryo quality. The main findings of the present study were: (i) combined flushing of oviduct and uterine horns resulted in the double number of recovered embryos compared to solely flushing of the uterine horns (ii) the quality of blastocysts produced by *in vivo* culture is superior to IVP derived blastocysts but inferior to embryos collected by superovulation as manifested in terms of conventional cryopreservation.

Using 54 animals it was shown that *in vivo* culture can be successfully performed in the bovine oviduct. Moreover, the minimal invasive manipulation allowed the re-use of all recipients in animal breeding programs at the farms. On the contrary, surgical techniques of access to oviduct in cattle were described only experimentally for a couple of animals and can lead to fertility limiting adhesions (Greve et al., 1989).

The embryo recovery rate is mainly influenced by the recovery method. Non-surgical flushing of the uterine horns led to only 28.7% re-collected embryos. In contrast, Xu et al. (1987) surgically performed *in vivo* culture in one animal and succeeded in a recovery rate of 74% after flushing of the uterine horns. Regarding each individual animal in our study where embryos were re-collected from the uterine horns, also high collection rates have been observed which reflect their findings. However, the average collection rate based on 36 recipients suggests that embryo collection from solely flushing of the uterine horns does not maximize the efficiency of embryo re-collection. In accordance to latter fact, the combined flushing of oviduct and uterine horn resulted in the double number of embryos compared to non-surgical flushing of the uterine horns. This result is comparable with the recovery rates after *in vivo* culture of embryos in the ligated sheep oviduct, reporting collection rates from 53% to 85% (Enright et al., 2000; Lonergan et al., 2003b). Our own data emphasize that after *in vivo* culture a considerable high number of embryos remain in the oviduct. Interestingly, the recovery rate was not affected by the embryonic stage and day of transfer within the same recovery system. With regard to the physiology of embryo transportation it is assumed that the reason for tubal embryo retention could be found in transport disturbances of the transferred embryos at the ampullary-isthmic region. Once the embryos reached this region a timely embryo migration towards the uterine horn is thought to be commenced (Greve and Callesen, 2001). Embryo migration in the oviduct is regulated by tubal peristalsis, ciliary activity and fluid currents influenced by endocrine and neural mechanisms. Thirty hours after ovulation the embryos arrive at the ampullary-isthmic junction and enter the uterus 72 h after ovulation at the 8- to 16-cell stage (El-Banna and Hafez, 1970). A maximum of tubal activity is expected around oestrogen dominance, which is abated during the next 2 to 5 days (Bennett et al., 1988; Killian et al., 1989). In accordance with our results Newcomb and Rowson (1975) found that five days after oestrus nearly half of the animals still had some eggs within the oviduct.

The total blastocyst rate at day seven and day eight (14% und 20%, respectively) in the bovine species was lower compared to ovine *in vivo* culture in the ligated oviduct (16% to 34%) (Enright et al., 2000; Rizos et al., 2002). Regarding the blastocyst rates of the present study it is clearly shown that the more the embryo cleavage is progressed before transfer the more blastocysts could be obtained after *in vivo* culture. But there is also evidence that embryo migration directly after transfer has a tremendous influence on blastocyst yield: our data suggest that if embryos were successfully transferred into the oviduct and underwent normal cleavage up to the blastocyst stage they mainly remained in the oviduct. Embryos found in the uterine horns obviously did not develop equally, neither on day seven nor on day eight. This aspect may be addressed to an early entry of tubal stage embryos into the uterine horns (Greve and Callesen, 2001). Consequently, the time of transfer obviously seems to have no effect on migration of the transferred complexes but a crucial effect on migration of the developmentally competent embryo.

The assessment of embryo quality as measured by cryotolerance demonstrated that embryos obtained by *in vivo* culture are in-between those produced

in vitro or collected after superovulation. Studies conducted in the ovine oviduct emphasized that *in vivo* cultured embryos showed survival rates comparable to embryos delivered by superovulation. These results confirmed the benifical effect of the tubal environment on the quality of IVM/IVF blastocysts (Enright et al. 2000; Rizos et al., 2002). Lonergan et al. (2003b) stated more precisely that the duration of *in vivo* culture is direclty related to embryo quality. In our study the limited time of embryo culture in the bovine oviduct could be a reason for the slight difference in embryo quality. Beside this sensitivity to cryopreservation differences between embryos cultured *in vivo* and *in vitro* can be detected by gene expression patterns (Wrenzycki et al., 1996; Lonergan et al., 2003a,b). These discrepancies in the transcription may be evident within as little as 10 hours of initiation of culture and in all cases are still evident at the blastocyst stage (Lonergan et al., 2003a). Moreover, these altered gene regulation and transcription activities may also reflect the morphological features at ultrastructural level between embryos cultured *in vivo* and *in vitro* (Crosier et al., 2001; Fair et al., 2001).

In conclusion, the results of the present study underline the successful culture of bovine embryos in the homologous oviduct resulting in a better embryo quality. It is emphasized, that use of *in vivo* culture for the generation of cloned offspring could have a beneficial effect. *In vivo* culture in the bovine oviduct requires embryo re-collection by combined flushing of oviducts and uterine horns. Further investigations are necessary to gain more detailed information about embryo quality, migration and embryo-maternal communication.

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