Acta vet. scand. 2003, *44,* 87-95.

# No Peri- and Postnatal Effects on Calves Born After Transfer of in Vitro Produced Embryos Vitrified by the Open Pulled Straw (OPS) Method

By *H. Jacobsen*[1], *P. Holm*[2], *M. Schmidt*[3], *B. Avery*[3], *T. Greve*[3] and *H. Callesen*[2]

[1]Institute of Food Safety and Nutrition, Danish Veterinary and Food Administration, Mørkhøj, [2]Department of Animal Breeding and Genetics, Danish Institute of Agricultural Sciences, Tjele and [3]Department of Clinical Studies, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

**Jacobsen H, Holm P, Schmidt M, Avery B, Greve T, Callesen H: No peri- and postnatal effects on calves born after transfer of in vitro produced embryos vitrified by the open pulled straw (OPS) method. Acta vet. scand. 2003, 44, 87-95.** – The general objective of this study was to perform follow-up studies including selected peri- and postnatal characteristics on calves born after transfer of in vitro produced (IVP) embryos vitrified by the 'Open Pulled Straw' (OPS) method. An overall pregnancy rate of 16% after transfer of the OPS-vitrified IVP embryos was achieved and resulted in birth of 9 calves, with 11 AI calves serving as controls. There were no immediate or long-term effects on these calves with respect to birth weight, gestation length, perinatal mortality, growth rate, disease susceptibility and reproductive performance.

***IVP calves; OPS vitrification; long-term studies; disease susceptibility; growth rates; reproductive status.***

## Introduction

In vitro produced (IVP) bovine embryos are more sensitive to chilling, cryopreservation and warming than their in vivo counterparts (*Leibo & Loskutoff* 1993, *Wurth et al.* 1994). One possible explanation for this difference could be a higher content of intracellular lipid concentration and a different zona pellucida in IVP embryos (*Leibo & Loskutoff* 1993). Culture medium containing serum probably contributes to a higher lipid concentration (*Gardner et al.* 1994, *Pollard & Leibo* 1994, *Shamsuddin & Rodriguez-Martinez* 1994, *Van Soom et al.* 1996, *Thompson* 1997, *Holm & Callesen* 1998). It has been suggested, that the increased lipid content may be a result of inefficient metabolism of lipid by mitochondria, which are fewer in IVP embryos (*Farin et al.* 2001). Culture without serum may improve viability following cryopreservation and may make the embryo more resamble the morphological appearance of in vivo embryos (*Thompson* 1997). However, in order for IVP embryos to be widely used, cryopreservation of these embryos and obtaining satisfactory pregnancy rates after transfer is essential. Some authors have shown comparable pregnancy rates after transfer of fresh and cryopreserved IVP embryos (*Wagtendonk-de Leeuw et al.* 1998, *Agca et al.* 1998) whereas other studies have resulted in a decreased pregnancy rates using cryopreserved IVP embryos (*Wurth et al.* 1994, *Van Soom et al.* 1994, *Hasler et al.* 1995).

The method of cryopreservation seems to have an impact on both rate of in vitro survival of IVP embryos and subsequent pregnancy. Vitrification seems to be superior to conventional

freezing both in vitro and after transfer (*Wurth et al.* 1994, *Dinnyés et al.* 1996, *Agca et al.* 1998). Vitrification by the so-called Open Pulled Straw (OPS) method is a promising method yielding in vitro hatching rates of Days 6-7 blastocysts of 94% and 70% at 48 and 72 h after warming, respectively (*Vajta et al.* 1998), but there are only a few studies regarding pregnancy rates following transfer of embryos vitrified by the OPS method. Lewis et al. (1999) obtained a pregnancy rate of 64% and a calving rate of 50% while 2 other studies found a pregnancy rate of 50% (*Holm et al.* 1999, *Lazar et al.* 2000).

After transfer, both fresh and frozen-thawed IVP embryos deviate from their in vivo counterparts in other aspects than lower pregnancy rates. There is a higher incidence of early embryonic loss and abortion throughout pregnancy, higher incidence of hydrallantois, deviation in vascularisation of the placenta, prolonged pregnancies, increased birth weight and a higher perinatal mortality of calves (*Van Soom et al.* 1994, *Hasler & al.* 1995, *Behboodi et al.* 1995, *Farin and Farin* 1995, *Sinclair et al.* 1995, *Schmidt et al.* 1996, *Kruip & den Daas* 1997, *Wagtendonk-de Leeuw et al.* 1998, 2000, *Hasler* 1998, *Numabe et al.* 1999, *Peterson et al.* 2000, *Numabe et al.* 2000, *Jacobsen et al.* 2000a, b, *Farin et al.* 2001). These problems related to the pregnancy and to the newborn calf have been denominated 'large offspring syndrome' (*Leese et al.* 1998, *Young et al.* 1998). There are only a few reports on follow-up studies caused by the in vitro procedure (*Wagtendonk-de Leeuw et al.* 2000).

The general objective of this study was to perform follow-up studies including growth rates, disease susceptibility and reproductive performance on calves born after transfer of OPS-vitrified IVP embryos as compared to AI control calves.

## Materials and methods

*Oocyte collection*

Ovaries from Holstein-Friesian cows and heifers were collected from a local abattoir and transported to the laboratory in physiological saline solution at 30-35 °C.

*Experimental group OPS-IVP-1*

The ovaries originated from culled cows and heifers from the research herd with known genetic background. The cows were fattened prior to slaughter. The oocytes were obtained by slicing the ovaries as described by *Hamano & Kuwayama* (1993), using a cutting device consisting of 10 parallel razor blades 2 mm apart in Hepes-buffered TCM 199 medium supplemented with 5% cattle serum.

*Experimental groups OPS-IVP-2 & OPS-IVP-3*

The oocytes were obtained by aspiration of 2 to 6 mm follicles from ovaries originating from Holstein-Friesian cows with unknown genetic, nutritional and reproductive status.

*In vitro maturation, fertilization and culture for experimental groups OPS-IVP-1 & -2*

Oocytes were matured and fertilized as described in detail by *Vajta et al.* (1996) and cultured as described by *Holm et al.* (1999). Briefly, groups of 25 to 30 cumulus-oocyte complexes were transferred to each well of a 4-well dish (Nunc, Roskilde, Denmark) containing 400 $\mu$l of TCM-199 medium (Gibco BRL, Paisley, UK) containing 25 mM bicarbonate, 0.2 mM sodium pyruvate, 0.4 mM L-glutamine, 50 $\mu$g/ml gentamycin, adjusted to pH 7.4 and 280 mOsm supplemented with 15% cattle serum as well as 10 IU/ml pregnant mare serum gonadotropin and 5 IU/ml human chorionic gonadotropin (Suigonan Vet, Intervet Scandinavia, Skovlunde, Denmark). The oocytes were incubated under paraffin oil (Uvasol 1.07161.0500, Merck, Darmstadt, Ger-

many) for 24 h at 39 °C in 5% CO2 in humidified air.

In vitro fertilization (Day 0) was performed with frozen-thawed semen from one Danish Holstein-Friesian bull. Oocytes were transferred to another 4-well dish containing 400 µl per well of IVF-TALP medium (*Parrish et al.* 1984, 1986, 1989) supplemented with 30 $\mu$g/ml heparin, 30 $\mu$M penicillinamine, 15 $\mu$M hypotaurine and 1 $\mu$M epinephrine and covered with paraffin oil. The semen was subjected to a 55% and 90% discontinuous Percoll (Phamacia, Uppsala, Sweden) gradient followed by 2 washes in sperm-TALP medium. Spermatozoa were added to the oocytes to give a final concentration of $1.5\times10^6$ spermatozoa per ml, and co-incubated at 39 °C under 5% $CO_2$ in humidified air.

Presumptive zygotes were cultured in modified SOFaa with 5% cattle serum under paraffin oil for 7 days at 39 °C in 5% $O_2$, 5% $CO_2$, and 90% $N_2$ in humidified air, with change of media on Day 4.

*In vitro maturation, fertilization and culture for experimental group OPS-IVP-3*

Oocytes were matured, fertilized and cultured as described in detail by *Avery et al.* (1998). Briefly, the maturation medium consisted of TCM 199 medium with Earle's salt and 25 mM sodium bicarbonate, supplemented with 10% oestrous cow serum, 1 mM L-glutamine, 0.2 mM sodium pyruvate, 1.4 IU/ml pregnant mare serum gonadotropin and 0.7 IU/ml human chorionic gonadotropin (Suigonan Vet, Intervet Scandinavia, Skovlunde, Denmark), 50 ng/ml epidermal growth factor (EGF) and 50 $\mu$g gentamycin.

In vitro fertilization (Day 0) was performed with frozen-thawed semen from the same bull used in experiment OPS-IVP-1 and -2. Oocytes were placed in 500 $\mu$l IVF-TALP medium, which contained 30 $\mu$g/ml heparin, 20 $\mu$M penicillamine, 10 $\mu$M hypotaurine, 1 $\mu$M epinephrine and 6 mg/ml bovine serum albumin. Frozen-thawed semen was washed twice in sperm-TALP medium and added to the oocytes in the fertilization medium to give a final concentration of $2\times10^6$ spermatozoa per ml and co-incubated for 20 h at 39 °C under 5% $CO_2$ in humidified air.

Presumptive zygotes were co-cultured with bovine oviduct cells in Menezo-B2 medium (INRA, Paris, France) supplemented with 10% estrous cow serum in 100 ml oil covered droplets (20 oocytes per droplet) at 39 °C under 5% $CO_2$ in humidified air. The embryos were cultured until Day 8 after insemination without change of media.

*Vitrification*

On Day 7, all embryos were evaluated using standard morphological criteria under a stereo microscope, and only blastocysts of excellent quality were selected for vitrification by the Open Pulled Straw (OPS) method as described in detail by *Vajta et al.* (1998). Briefly, the middle section of French mini straws (250 $\mu$l; IMV, L'Aigle, France) were heat-softened over a hot plate before being pulled manually until the inner diameter and the wall thickness decreased from 1.7 mm to approximately 0.8 mm and from 0.15 mm to approximately 0.07 mm, respectively. The straws were cooled in air, then cut at the narrowest point with a razor blade. For vitrification, each blastocyst was first incubated in holding medium (TCM-Hepes supplemented with 20% cattle serum) containing 7.5% ethylene glycol and 7.5% dimethyl sulphoxide for 3 min, and then transferred in approximately 1 to 2 $\mu$l of solution to a 20-$\mu$l droplet holding medium containing 16.5% ethylene glycol, 16.5% dimethyl sulphoxide and 0.5 M sucrose. The blastocyst and the solution was mixed quickly by pipetting and then transferred into another drop containing ap-

proximately 1 to 2 $\mu$l solution. Loading of the blastocysts into the straw was performed using the capillary effect by simply touching the 1 to 2 $\mu$l droplet, containing the blastocysts with the narrow end of the pulled straw. This end was then immediately submerged into liquid nitrogen. Warming was performed by immersing the end of the straw containing the blastocysts into 1.2 ml of holding medium containing 0.25 M sucrose. After 1 min, the blastocysts were transferred into 1.2 ml of holding medium containing 0.12 M sucrose for another 5 min and then twice for 5 min. Finally the blastocysts were transferred twice for 5 min each into holding medium. The temperature of all media was 37 °C. Subsequently, the blastocysts were cultured in SOFaa with 5% cattle serum for 4 h prior to transfer as described for culture of OPS-IVP-1 and -2.

*Embryo transfer*

Experimental group OPS-IVP-1. From December 1997 to February 1998, 32 cows had a single embryo non-surgically transferred on Days 6-8 after external signs of heat. The oestrus period used was the first spontaneous one postpartum that otherwise would have been used for insemination. Pregnancy diagnosis was carried out on Day 45 by rectal palpation and ultrasound scanning.

Experimental groups OPS-IVP-2 and -3. Forty-four heifers were heat synchronized 11 days apart with an i.m. injection of a synthetic $PGF_{2\alpha}$ analogue (0.5 mg cloprostenol, Estrumat, Schering-Plough, Farum, Denmark). Twenty-eight and 16 heifers had one embryo from experimental groups OPS-IVP-2 and -3, respectively, non-surgically transferred 7 days after external signs of heat from April to July 1998. Pregnancy diagnosis was carried out on Day 45 by rectal palpation and ultrasound scanning.

*Control group*

Twenty-four cows were inseminated with semen from the same bull used for IVF and were examined for pregnancy on Day 45 by rectal palpation and ultrasound scanning.

*Delivery and postnatal examination of all the calves*

All recipients and control cows were closely observed during the deliveries that occurred from September 1998 to April 1999. If obstetrical assistance was required, it was not applied until 2½-3 h after rupture of the fetal membranes (allanto-amnion). Immediately after birth, nasal passages were cleared and navels were sprayed with iodine solution. Thereafter, the calves were weighed and then transported to separate calf pens, where they were fed 40 ml/kg colostrum within the first 2 h after birth. Calves were housed in separate calf pens for 1½ to 2 months and then moved to large calf pens with 5-8 calves per pen. These calf pens were placed in one of the cow stalls. During winter and early spring the cows were inside at all times whereas the cows were on pasture during the day in late spring and summer.

All calves were routinely weighed and growth rates were calculated as daily weight gain. The anticipated growth rate was 0.6 kg per day for heifer calves and 1.3 kg per day for bull calves from Day 123. Bull calves were either sold at 6 to 7 months of age or slaughtered at 10 months of age.

The health of the calves was carefully monitored and any diseases and treatments were recorded.

When heifers reached a weight of between 250 and 300 kg they were observed for external signs of heat. It was intended the heifers should calve at 24 to 26 months of age, and therefore inseminations were begun at 15 to 17 months of age. Heat observations, inseminations and pregnancy status were recorded.

Table 1. Number of embryos transferred, pregnancy rates and number of calves born following transfer of OPS-vitrified embryos or AI.

| Experimental group | No. of single embryo transferred | Pregnancy rate (pregnant/ transfer) | No. of calves born |
|---|---|---|---|
| IVP-1 | 32 | 9.4% (3/32) | 3 |
| IVP-2 | 28 | 21.4% (6/28) | 5 |
| IVP-3 | 16 | 18.9% (3/16) | 1 |
| Total-IVP | 76 | 15.8% (12/76) | 9 |
| Control-AI | 24 inseminations | 50% (12/24) | 11 |

*Statistical methods*

Data were analyzed by a mixed linear model (Proc Mixed, SAS 1992), and results presented as LSmeans ± SEM. Birth weights were analyzed using a model in which embryo treatment (IVP or AI), sex and gestation length was used as fixed effects. For gestation length, embryo treatment (OPS-IVP or AI) and sex were used as fixed effects. For growth rate, female and male calves were analyzed separately and then embryo treatment was used as a fixed effect. Differences with P-values <0.05 were regarded as statistically significant.

## Results

*Pregnancy rates and number of calves*

Pregnancy rates and number of calves are shown in Table 1. Abortions occurred for 1 heifer in the OPS-IVP-3 group at 7 months of pregnancy, for 1 heifer in the OPS-IVP-2 group at 3½ months and for 1 AI cow at 6 months of pregnancy. Lightening killed one pregnant heifer in the OPS-IVP-3 group.

*Dystocia, birth weight and gestation length*

All 11 AI and 3 OPS-IVP-1 calves were born by cows, whereas 5 OPS-IVP-2 and 1 OPS-IVP-3 calves were born by heifers.

Of the 6 heifers calving, 4 needed assistance during delivery, whereas none of the cows needed assistance.

Table 2 shows birth weights, gestation length and sex ratio of the AI and OPS-IVP calves. There was no significant effect of embryo treatment (AI vs. OPS-IVP) on birth weight or gestation length. There was a positive correlation between gestation length and birth weight ($r = 0.57$, $p < 0.008$) and a significant effect of sex on birth weight, with male calves being heavier than female calves (LSmeans ± SEM, male: 50.1 ± 1.4 kg, female: 45.0 ± 1.2 kg, $p < 0.002$). Parity and weight of the recipient did not affect birth weight or gestation length in this study and therefore these parameters are not included in the statistical model.

The largest calf (female, AI, 56 kg) was found dead. Since its hair was not loose it was concluded that the calf died during delivery or im-

Table 2. Birth weight (LSmeans ± SEM, kg), gestation length (LSmeans ± SEM, days), and sex ratio in total IVP- and control AI calves.

| Experimental group | Birth weight (range) | Gestation length (range) | Male /female |
|---|---|---|---|
| Total-IVP (n = 9) | 44. 2 ± 0.9 kg (40-48) | 280.0 ± 1.5 days (272-286) | 4 / 5 |
| Control-AI (n=11) | 46.6 ± 0.8 kg (38-56) | 279.1 ± 1.4 days (267-287) | 4 / 7 |

mediately thereafter. One of the OPS-IVP recipient heifers showed weak labour and deficient dilatation of the cervix. A Cesarean section was performed, but the bull calf was found dead; since its hair was loose it was concluded that it had died at least 12 h earlier. One female OPS-IVP calf broke its leg when it was 2 weeks old and was consequently euthanised.

*Survival, disease susceptibility, growth and reproductive status*

Of the remaining 10 AI and 7 OPS-IVP calves, susceptibility to diseases was not related to the AI or OPS-IVP procedures, but was dependent on the time of year when the calves were born and also to the type of housing. In January 1999 an outbreak of diarrhoea affected all calves born (10 AI and 2 OPS-IVP) and 3 AI calves were treated for pneumonia. In March and April 1999 pneumonia affected most of the calves and the herd veterinarian treated 3 AI and 2 OPS-IVP calves. One female AI calf never recovered from pneumonia and diarrhoea and was euthanised at 3 months of age. Two of the OPS-IVP calves born in spring were treated for pneumonia in July 1999.

The average growth rate per day of heifers was not different between the AI and OPS-IVP groups (means ± SEM kg/day; AI: 0.56 ± 0.01, n = 5; OPS-IVP: 0.58 ± 0.05, n = 4; $p > 0.05$). All bull calves in both the AI and OPS-IVP groups had a growth rate from Day 123 above the target level of 1.3 kg/day (means ± SEM kg/day; AI: 1.52 ± 0.12; OPS-IVP: 1.46 ± 0.07, $p > 0.05$).

The first heat was registered at an average age of 16.5 months for both the 4 OPS-IVP heifers (15-20.5 months) and the 5 AI heifers (15.5-18 months). All heifers are currently diagnosed pregnant, the 4 OPS-IVP heifers at an average age of 17.5 months (15-20.5 months) with an average of 2.0 ± 0.6 inseminations per pregnancy, and the 5 AI heifers at an average age of 18 months (15.5-20 months) with an average of 2.4 ± 0.7 inseminations per pregnancy.

**Discussion**

Although the study is based on a limited animal material it showed that there was no immediate effect on pregnancy and calves born after in vitro embryo production including the OPS-vitrification with respect to gestation length, birth weight or perinatal mortality compared with AI calves. Similarly there was no long-term effect of the in vitro procedure including the OPS-vitrification on growth rate, diseases susceptibility or reproductive performance of the heifer calves.

Pregnancy rates following transfer of the OPS-vitrified IVP embryos were lower compared with previous studies using similar types of embryos (*Holm et al.* 1999, *Lewis et al.* 1999, *Lazar et al.* 2000). Previous experiments in the same herd using the same IVP and vitrification procedure have also given relatively low pregnancy rates (18%, *Callesen,* personal communication). Whether the low pregnancy rates in this study were a result of poor recipients, the IVP-procedure, the OPS-vitrification or other parameters remains unknown.

There may be several reasons for only obtaining a 9.4% pregnancy rate in the IVP-1 experimental group. One reason may be the oocyte quality, because the ovaries originated from fattened cows that were intended for culling. Consequently their nutrition might have reduced oocyte quality with a lasting influence on the developmental competence of the resulting embryos (*Blanchard et al.* 1990). In addition, the oocytes were obtained by slicing the ovaries which is a procedure known to give a very heterogeneous population of oocytes including those with poor developmental competence (*Fair et al.* 1995, 1996). A Second reason may be recipient type; the embryos were transferred to cows 6-8 days after the first spontaneous heat

postpartum that would otherwise have been used for insemination. In addition, the cows were not selected on the basis of the presence of a corpus luteum, but were selected solely on basis of the information that they had been observed in heat. A third reason could have been the synchrony between the embryo and the recipient since the embryos were transferred on days 6-8 after external signs of heat and consequently could have been transferred to an asynchronous environment. However, the results from a large Danish field trial on embryo transfer of in vivo produced embryos established that while asynchrony of ± 1 day had no effect on pregnancy rates, ± 2 days did (*Liboriussen & Andersen* 1997).

The pregnancy rate did improve from 10% to 20% when heat synchronized heifers were used as recipients. In a previous small study using the same procedures for IVP and vitrification, *Holm et al.* (1999) obtained a pregnancy rate of 50% (4/8). This indicates that the procedure is also viable under practical conditions.

There was no long-term effect of the IVP procedure including OPS-vitrification on the calves. Growth rates were similar between AI and OPS-IVP heifers and in both groups, only slightly lower than the expected 0.6 kg/day. Bull calves had growth rates above the expected 1.3 kg/day from day 123, which was considered satisfactory. Regarding disease susceptibility, the AI calves seemed more susceptible than the OPS-IVP calves. However, diarrhoea and pneumonia went through the herd in January 1999 and affected most calves at a time when all AI control calves and only 2 of the 7 OPS-IVP calves were born. Therefore, we conclude that the time of year when the calves were born was more important than the origin of the embryo regarding disease susceptibility.

For OPS-IVP embryos to be a useful and competitive technology in comparison to in vivo embryo production by superovulation, the negative effects of the 'large offspring syndrome' have to be overcome and it is also necessary to obtain higher pregnancy rates after cryopreservation. In the present study there were no immediate or long-term effects of the IVP procedure including OPS-vitrification on the calves which is certainly a positive result. However, a pregnancy rate of 16% was not satisfactory, and in the future the quality of the ovaries and the recipients should be more carefully selected. In addition to the AI control group we should have had a control group of recipients receiving fresh IVP embryos in order to differentiate whether the poor pregnancy result was due to the vitrification procedure or the IVP procedure.

## Conclusions

It is concluded that a pregnancy rate of 16% after transfer of OPS-vitrified embryos in this experiment was disappointedly low. However, there was no immediate or long-term effect on the calves of our standard IVP system including OPS-vitrification with respect to birth weight, gestation length, perinatal mortality, growth rate, disease susceptibility and reproductive performance.

## References

*Agca Y, Monson RL, Northey DL, Mazni OA, Schaefer DM, Rutten VP:* Transfer of fresh and cryopreserved IVP bovine embryos: Normal calving, birth weight and gestation lengths. Theriogenology. 1998, *50,* 147-162.

*Avery B, Hay-Schmidt A, Hyttel P, Greve T:* Embryo development, oocyte morphology, and kinetics of meiotic maturation in bovine oocytes exposed to 6-dimethylaminopurine prior to in vitro maturation. Mol.Reprod.Dev. 1998, *50,* 334-344.

*Behboodi E, Anderson RH, BonDurant RH, Gargill SL, Kreusher BR, Medrano JF, Muurrah JD:* Birth of large calves that developed from in vitro derived bovine embryos. Theriogenology. 1995, *44,* 227-232.

*Blanchard T, Ferguson J, Love L, Takeda T, Henderson B, Hasler J, Chapula W:* Effect of dietary crude-protein on fertilization and embryo quality

in dairy cattle. Am.J.Vet.Res. 1990, *51,* 905-908.

*Dinnyés A, Carolan C, Lonergan P, Massip A, Mermillod P:* Survival of frozen or vitrified bovine blastocysts produced in vitro in synthetic oviduct fluid. Theriogenology. 1996, *46,* 1425-1439.

*Fair T, Hyttel P, Greve T:* Bovine oocyte diameter in relation to maturational competence and transcriptional activity. Mol.Reprod Dev. 1995, *42,* 437-442.

*Fair T, Hyttel P, Greve T, Boland M:* Nucleus structure and transcriptional activity in relation to oocyte diameter in cattle. Mol.Reprod Dev. 1996, *43,* 503-512.

*Farin PW, Farin CE:* Transfer of bovine embryos produced in vivo or in vitro: survival and fetal development. Biol.Reprod. 1995, *52,* 676-682.

*Farin PW, Grosier AE, Farin CE:* Influence of in vitro system on embryo survival and fetal development in cattle. Theriogenology. 2001, *55,* 151-170.

*Gardner DK, Lane M, Spitzer AR, Batt RM:* Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage in vitro in the absence of serum and somatic cells: amino acids, vitamins, and culturing embryos in groups stimulate development. Biol. Reprod. 1994, *50,* 390-400.

*Hamano S, Kuwayama M:* In vitro fertilization and development of bovine oocytes recovered from the ovaries of individual donors: A comparison between the cutting and aspiration method. Theriogenology. 1993, *39,* 703-712.

*Hasler J:* The current status of oocyte recovery, in vitro embryo production, and embryo transfer in domestic animals with an emphasis on the bovine. J.Anim.Sci. 1998, *76 (suppl 3),* 52-74.

*Hasler J, Henderson W, Hurtgen J, Jin Z, McCauley A, Mower S, Neerly B, Shuey L, Stokes J, Trimmer S:* Production, freezing and transfer of bovine IVF embryos and subsequent calving results. Theriogenology. 1995, *43,* 141-152.

*Holm P, Callesen H:* In vivo versus in vitro produced bovine ova: Similarities and differences relevant for practical application. Reprod.Nutr.Dev. 1998, *38,* 579-594.

*Holm P, Booth P, Schmidt MH, Greve T, Callesen H:* High bovine blastocyst development in a static in vitro production system using SOFaa medium supplemented with sodium citrate and myo-inositol with or without serum proteins. Theriogenology. 1999, *52,* 683-700.

*Holm P, Vajta G, Booth P, Callesen H:* OPS-vitrification versus conventional ethylene glycol freezing of bovine IVP-blastocysts: In vitro kinetics of re-expansion and hatching. Proc.15th Scientific meeting of A.E.T.E., Lyon 10-11 September. 1999, p 168.

*Jacobsen H, Schmidt M, Holm P, Sangild PT, Greve T, Callesen H:* Ease of calving, blood chemistry, insulin and bovine growth hormone of newborn calves derived from in vitro produced embryos cultured with or without serum and oviduct epithelium cells. Theriogenology. 2000a, *54,* 147-158.

*Jacobsen H, Schmidt M, Holm P, Sangild PT, Vajta G, Greve T, Callesen H:* Body dimensions and birth and organ weights of calves derived from in vitro produced embryos cultured with or without serum and oviduct epithelium cells. Theriogenology. 2000b, *53,* 1761-1769.

*Kruip TAM, den Daas JH:* In vitro produced and cloned embryos: Effects on pregnancy, parturition and offspring. Theriogenology. 1997, *47,* 43-52.

*Lazar L, Spak J, David V:* The vitrification of in vitro fertilized cow blastocysts by the Open Pulled Straw method. Theriogenology. 2000, *54,* 571-578.

*Leese HJ, Donnay I, Thompson JG:* Human assisted conception: a cautionary tale. Lessons from domestic animals. Hum.Reprod. 1998, *13 Suppl 4,* 184-202.

*Leibo SP, Loskutoff NM:* Cryobiology of in vitro-derived bovine embryos. Theriogenology. 1993, *39,* 81-94.

*Lewis IM, Lane MW, Vajta G:* Pregnancy rates following transfer of in vitro produced bovine embryos vitrified by the open pulled straw (OPS) method. Theriogenology 51, p.168 1999, abstract.

*Liboriussen T, Andersen BB (eds):* Embryo transfer, physiological challenge test and nucleus breeding with dairy cattle. Danish Institute of Agricultural Sciences, 1997, Internal Report No 737, 116 pp.

*Numabe T, Oikawa T, Kikuchi T, Horiuchi T:* Birth weights of calves conceived by transfer of Japanese black cow embryo produced in vitro by co-culture with cumulus cells. Theriogenology. 1999, 51, 327 (abstract).

*Numabe T, Oikawa T, Kikuchi T, Horiuchi T:* Birth weight and birth rate of heavy calves conceived by transfer of in vitro or in vivo produced bovine embryos. Anim Reprod Sci. 2000, *64,* 13-20.

*Parrish JJ, Parrish JL, First NL:* Effect of swim-up separation and heparin pretreatment of frozen-

thawed spermatozoa on in vitro fertilization of bovine oocytes. Biol.Reprod 30 (suppl 1), p.211 1984, abstract.

*Parrish JJ, Susko-Parrish JL, First NL:* Capacitation of bovine sperm by heparin: inhibitory effect of glucose and role of intracellular pH. Biol.Reprod. 1989, *41,* 683-699.

*Parrish JJ, Susko-Parrish JL, Leibfield-Ruthledge ML, Critse ES, Eyestone W, First NL:* Bovine in vitro fertilization with frozen-thawed semen. Theriogenology. 1986, *25,* 591-600.

*Peterson AJ, McMillan WH, Thompson JG:* Varios allantoic pathologies are associated with malformation of allantoic development of the IVP embryo. Proc. 14'th ICAR. 2000, *1,* 158 (abstract).

*Pollard JW, Leibo SP:* Chilling sensitivity of mammalian embryos. Theriogenology. 1994, *41,* 101-106.

*SAS.* SAS Technical Report P229, SAS/STAT Software: Changes and Enhancement. Release 6.07. Cary NC: Statistical Analysis Institute INC. 1992; p.287

*Schmidt MH, Greve T, Avery B, Beckers JF, Sulon J, Hansen HB:* Pregnancies, calves and calf viability after transfer of in vitro produced bovine embryos. Theriogenology. 1996, *46,* 527-539.

*Shamsuddin M, Rodriguez-Martinez H:* Fine structure of bovine blastocysts developed either in serum-free medium or in conventional co-culture with oviduct epithelial cells. J.Vet.Med.A. 1994, *41,* 307-316.

*Sinclair KD, Broadbent PJ, Dolman DF:* In vitro produced embryos as a means of achieving pregnancy and improving productivity in beef cows. J.Anim Sci. 1995, *60,* 55-64.

*Thompson JG:* Comparison between in vivo-derived and in vitro-produced pre-elongation embryos from domestic ruminants. Reprod Fertil.Dev. 1997, *9,* 341-354.

*Vajta G, Holm P, Greve T, Callesen H:* Overall efficiency of in vitro embryo production and vitrification in cattle. Theriogenology. 1996, *45,* 683-689.

*Vajta G, Holm P, Kuwayama M, Booth PJ, Jacobsen H, Greve T, Callesen H:* Open Pulled Straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos. Mol.Reprod Dev. 1998, *51,* 53-58.

*Van Soom A, Bols PEJ, Vanroose G, Kruif A:* A comparison between in vivo and in vitro produced bovine embryos: scientific and practical application. Proc. 12th ICAR 1996, *3,* 1365-1367.

*Van Soom A, Mijten P, Van Vlaenderen I, Van den Branden J, Mahmoudzadeh AR, Kruif A:* Birth of double-muscled Belgian blue calves after transfer of in vitro produced embryos into dairy cattle. Theriogenology. 1994, *41,* 855-867.

*Wagtendonk-de Leeuw AM, Aerts BJ, den Daas JH:* Abnormal offspring following in vitro production of bovine preimplantation embryos: a field study. Theriogenology. 1998, *49,* 883-894.

*Wagtendonk-de Leeuw AM, Mullaart E, de Roos AP, Merton JS, den Daas JH, Kemp B, de Ruigh L:* Effects of different reproduction techniques: AI MOET or IVP, on health and welfare of bovine offspring. Theriogenology. 2000, *53,* 575-597.

*Wurth YA, Reinders JMC, Rall WF, Kruip TAM:* Developmental potential of in vitro produced bovine embryos following cryopreservation and single-embryo transfer. Theriogenology. 1994, *42,* 1275-1284.

*Young LE, Sinclair KD, Wilmut I:* Large offspring syndrome in cattle and sheep. Rev.Reprod. 1998, *3,* 155-163.

## Sammendrag

*Ingen peri eller postnatal effekt på kalve født efter transplantation af in vitro producerede embryoner vitrificeret ved hjælp af OPS-vitrification.*

Målet med dette studie var at undersøge, om der var en langtidseffekt på kalvene af in vitro producerede embryoner, der var blevet vitrificeret ved hjælp af 'Open Pulled Straw' (OPS) metoden. Langtidsstudierne inkluderede tilvækst, sygdomsforekomst og reproduktionsstatus. Der blev opnået en overordnet drægtighedsprocent på 16% efter transplantation af OPS-vitrificerede IVP embryoner, hvilket var lavere end forventet. Dette resulterede i fødslen af 9 IVP kalve, og som kontrol fungerede 11 AI kalve. Der var ingen kort- eller langtidseffekt på kalve født efter transplantation af OPS-vitrificerede IVP embryoner mht. fødselsvægt, drægtighedslængde, perinatal dødelighed, tilvækst, sygdomsforekomst eller reproduktionsstatus.

*(Received January 10, 2001; accepted August 1, 2001).*

Reprints may be obtained from: T. Greve, Department of Clinical Studies, Royal Veterinary and Agricultural University, Dyrlægevej 68, 1870 Frederiksberg C, Copenhagen. E.mail: tg@kvl.dk, tlf. +45 35 28 29 65, fax: +45 35 28 29 72.