

Carbon-activated gas filtration during in vitro culture increased pregnancy rate following transfer of in vitro-produced bovine embryos

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Abstract

Many environmental conditions for in vitro embryo production (IVP) systems for cattle have been relatively standardised, e.g. media composition, temperature, pH, water quality and atmospheric composition. However, little attention has been paid to the quality of ambient laboratory air and the gas environment in incubators. Although a few studies have examined the effects of chemical air contamination on IVP of human embryos, there are no published accounts for domestic animal embryos. Therefore, this study investigated the effects of an intra-incubator carbon-activated air filtration system (CODA), during in vitro culture (IVC) on embryonic development and subsequent pregnancy rate of bovine embryos. Immature Cumulus-Oocyte-Complexes (COCs) were obtained twice weekly by ultrasonic guided transvaginal oocyte aspiration. COCs were matured in TCM199/FCS/LH/FSH, fertilized with frozen-thawed Percoll-separated semen, and subsequently cultured for 7 days in SOFaaBSA. Day 7 embryos were transferred either fresh or frozen/thawed. The experimental design was a 2X2 factorial, whereby presumptive zygotes were placed either in a normal CO₂-O₂-N₂ incubator (Control group) or in an identical CO₂-O₂-N₂ incubator with a CODA intra-incubator air purification unit (CODA group) for IVC. The embryo production rate at Day 7 was not affected by the CODA air purification unit (23.4% and 24.7% morulae and blastocysts per oocyte for control and CODA respectively). Also, there was no effect on embryo stage or quality. However, the pregnancy rate was improved (P=0.043) for both fresh (46.3 vs. 41.0%) and frozen/thawed embryos (40.8 vs. 35.6%). These results show that atmospheric purification by the CODA intra-incubator air purification unit significantly increased pregnancy rate following transfer of in vitro-produced bovine embryos.

Key words: IVP, Pregnancy rate, VOC

Introduction

Commercial embryo transfer in cattle has grown into a large, world-wide business over the past 35 years. Statistics compiled by the International Embryo Transfer Society (IETS) indicate that more than 550,000 in vivo-derived embryos, obtained primarily from superovulated cattle, were transferred internationally in 2004 (Thibier, 2005). Improvements over the past 15 years in procedures for in vitro production of cattle embryos have resulted in the establishment of

commercial in vitro-embryo transfer programs (Hasler, 1998). These programs include the salvage of genetics from infertile females, increased embryo production from reproductively-healthy females, and the large-scale production of embryos from slaughterhouse material. IETS statistics (Thibier, 2005) indicate that approximately 240,000 in vitro-produced embryos were transferred worldwide in 2004. The largest programs are in South America, where most embryos are derived from repeated ultrasound guided transvaginal oocyte collections, also known as ovum pick up (OPU) from living females, and Asia, where embryos primarily are produced from oocytes obtained from slaughterhouse-derived ovaries. In Europe, the use of OPU-IVP is limited. Only six countries reported producing IVP embryos in 2004 (Merton, 2005), due to the fact that OPU-IVP is mainly used for breeding purposes and not for commercial production as is common in other programs.

Many of the environmental conditions for in vitro embryo production, e.g. media composition, temperature, pH, water quality and atmospheric composition, have been relatively standardized in both human and animal in vitro production (IVP) systems. Less attention has been paid, however, to the quality of both ambient laboratory air and the gaseous atmosphere inside incubators.

Atmospheric particulate matter may affect pregnancy rates in human IVF programs. In a retrospective analysis of an ongoing program (Boone, et al., 1999), it was shown that pregnancy rates decreased substantially during a period of construction adjacent to the laboratory complex. Installation of ultra low penetration air (ULPA) filters resulted in a substantial improvement in pregnancy rates. In addition, a positive pressure differential system was installed in the entry and laboratory rooms. Following these changes, counts of $0.3 \mu\text{m}$ particles (particles counted for a specific length of time) ranged from less than 1 in the embryology laboratory rooms to over 450,000 in the adjacent operating room in which there was no ULPA filtration system.

In addition to physical particles, it is clear that chemical air contamination and volatile organic compounds (VOCs) impact laboratory and incubator environments and may affect IVP and/or subsequent pregnancy rate. Although many laboratories now have high efficiency particle air filtration (HEPA or ULPA) systems, these filters do not efficiently retain gaseous organic and inorganic molecules. The gaseous atmosphere in incubators often is a combination of room air, CO_2 and sometimes also N_2 from compressed gas tanks. All of these sources may contain contaminants. Schimmel et al., 1997, showed that among the various organic compounds found in laboratory gases, benzene was derived specifically from the CO_2 tanks. In some IVP systems, a high percentage (>90%) of ambient air in the incubator is obtained

directly from the laboratory room through the opened door or the inlet port at the back of the incubator. Surprisingly, Cohen et al., 1997, found that unfiltered, outside air may be cleaner than air both in the laboratory and in incubators. This can result from the accumulation of VOCs from the laboratory complex and from laboratory products such as plastic dishes. Refrigerant gases, isopropyl alcohol fumes, various aliphatic hydrocarbons and select aromatic compounds were found to be at higher levels in the laboratory air and inside the incubators compared to outside air. Cohen et al., 1997 also determined that a large number of VOCs were detectable in compressed CO₂ used for in vitro culture.

In general, air pollutants such as VOCs, small inorganic compounds (e.g. N₂O, SO₂ and CO), and heavy metals can be detected. Sources of these contaminants are VOCs produced by industrial and vehicle exhaust, but also by cleaning agents and off-gassing from plastic ware, media, laboratory furnishing and equipment (Hall, et al., 1998). Disposable plastic petri dishes and flasks have been shown by gas chromatography to emit a number of volatile organic compounds, including ethyl-benzene and benzaldehyde (Gilligan, et al., 1997).

To overcome possible effects of these contaminants on embryo production and/or subsequent pregnancy rate, some laboratories have placed filters containing activated carbon and potassium permanganate in the laboratory or inside the incubators, recycling the air while removing or diminishing toxic air pollutants. Mayer et al., 1999, examined the effect of CODA intra-incubator air filter on human embryo quality and pregnancy rates. Although there was no effect on the number and quality of embryos produced, pregnancy rate was significantly higher, 52% versus 30%, following transfer of embryos cultured in CODA-filtered versus non-filtered incubators.

Racowsky et al., 1999, also reported on human IVF-derived pregnancies resulting from embryos cultured in filtered air. Filtration of ambient, HEPA-filtered, laboratory air and incubator CO₂ gas by carbon-activated filters resulted in a slight but significant difference in embryo fragmentation and no significant difference in pregnancy rate, but a significant reduction in the spontaneous abortion rate. Most of the results reported in humans are based on small numbers of transfers, which makes the demonstration of potentially statistically significant but small differences more difficult.

This study was designed to investigate the effects of an intra-incubator carbon-activated air filtration system (CODA) during IVC of bovine in vitro matured (IVM) and fertilized (IVF) COCs on embryonic development and subsequent pregnancy rate.

Materials and Methods

Ovum Pick up and in vitro production of preimplantation embryos

From 27 March 2000 until 05 March 2001, OPU was conducted from 193 donors at 2 different locations in The Netherlands. Donors were 177 pregnant heifers and 16 first parity cows (Holstein Friesian) from the delta open nucleus breeding program of Holland Genetics. An average of 7.3 oocyte collections from pregnant heifers and an average of 30.3 collections from first parity cows were performed. Immature COCs were obtained twice weekly, on Mondays and Thursdays, by ultrasound-guided transvaginal oocyte collection. COCs were matured in TCM199/FCS/LH/FSH, fertilised with frozen-thawed Percoll-separated semen (Day 0), cultured for 6 days in SOFaaBSA as described previously (van Wagendonk, et al., 2000) and evaluated starting on day 7. The intra-incubator gas atmosphere for IVM and IVF was 5% CO₂, which was supplied from a compressed cylinder. For IVC, the atmospheric mixture was 5% CO₂, 5% O₂ and 90% N₂, with the CO₂ and N₂ provided by compressed cylinders.

Embryo transfer

Embryos were evaluated for stage and grade on days 7 and 8 (day 0 = day of IVF) by IETS standards (Robertson and Nelson, 1998). Depending on the number of transferable embryos available on Day 7 of culture (IETS stage 4-grade 1 and stage 5 to 9-grades 1 and 2) and the number of recipients available, embryos either were transferred fresh or frozen/thawed (IETS stage 5-grade 1 and stage 6 to 9-grades 1 & 2) using conventional slow freezing in 10% glycerol as previously described (van Wagendonk, et al., 1995). Fresh embryos were transported at 25C° in Emcare Holding Solution (ICP, New Zealand) to participating farms throughout The Netherlands and to recipient herds owned and managed by Holland Genetics. Recipients were evaluated by rectal palpation for the presence of a corpus luteum on day 7 or 8 of their natural estrous cycle. Potential recipients were rejected for obvious reasons such as endometritis or absence of a corpus luteum. Suitable recipients received an embryo at Day 7 or 8 by standard nonsurgical embryo transfer procedures. Pregnancy data were obtained at participating farms between days 90 and 280 of gestation.

Experimental design

The experimental design was a 2X2 factorial (OPU location and treatment) randomized crossover, whereby the presumptive zygotes were placed either in a normal CO₂-O₂-N₂ incubator (Control group) or in an identical CO₂-O₂ incubator with a CODA (<genX>

International, Inc., Madison, CT, USA) intra-incubator air purification unit (CODA group). Every five weeks the CODA intra-incubator air purification unit was transferred to the other incubator to eliminate any variables due to the performance of the specific incubators.

Statistical analyses

Results were analysed by Chi-square for embryonic development and logistic regression analysis for pregnancy rates.

Results

In vitro embryo production

A mean of 8.2 COCs per collection were recovered during 1,607 OPU sessions. As shown in Table 1, no difference was found between the control and CODA treatment groups for either cleavage (58.3 and 59.7%, respectively) or blastocyst formation at Day 8 (19.3 and 20.3%, respectively). In addition, the embryo production rate (morulae and blastocysts combined) was not affected on Day 7 by the CODA air purification unit (23.4% and 24.7% for control and CODA respectively). Also, within the population of embryos at Day 7, the CODA air purification unit had no effect on the distribution of embryos among the different grades (Figure 1) or stages (Figure 2) at Day 7.

Embryo transfer

From the total of 3151 embryos produced on Day 7, 1666 were classified as transferable quality (820 fresh and 846 frozen; 1.04 embryos per OPU session). Pregnancy data following transfer of 782 fresh and 635 frozen embryos were obtained and analysed (Table 2). There was a significant effect ($P = 0.043$) of the CODA air purification unit on pregnancy rate for both fresh and frozen/thawed embryos. The increase was 5.3 and 5.2 percentage points for fresh and frozen/thawed embryos respectively, which are increases of 12.9 and 14.6%.

Discussion

This study did not show an effect of intra-incubator gas purification by the CODA unit on embryos as judged by percent cleavage, embryo quality or stage or development. However, the pregnancy rate was significantly improved following transfer of both fresh and frozen/thawed

embryos produced in the CODA system. This suggests an improvement in the intrinsic quality of the embryo that was not detectable by morphology.

Similar results were reported for human embryos by Mayer et al., 1999. As in the present study, Mayer et al., 1999, did not find an effect on the number or quality of embryos produced. However, the pregnancy rate was significantly affected, suggesting an improved embryo quality that was not detectable by morphology. In a study by Racowsky et al., 1999, CODA-filtered incubation resulted in a significant decrease in the apparent morphological quality of embryos, as judged by fragmentation. The authors suggested that the increase in fragmentation might provide a means by which embryos may improve their developmental competency and that the use of CODA air purification units might facilitate this process. In a recent study involving human IVF in Brazil, in line HEPA and carbon-activated filters located between the gas cylinders and the incubators and intra-incubator filtration units resulted in higher cleavage rates, more good quality embryos, higher pregnancy rates and lower spontaneous abortion rates than in a control lab with a HEPA filtration system and a CODA Tower (Esteves, et al., 2004).

In another study, the effectiveness of HEPA filtration and high-activity charcoal and potassium permanganate filters for cleaning ambient laboratory air were further improved by the addition of a CODA Tower in the laboratory (Forman, et al., 2004). The improvement included decreases in the concentrations of VOC, aldehydes and particulates.

It is noteworthy that the improvement in embryo quality in the present study was achieved with CODA filtration during only six days of ICV, and filtration did not include the periods of IVM and IVF. It can be hypothesised that during this early phase of development, when the embryonic genome has to become activated, the atmospheric environment affects embryo quality in a subtle manner not detectable by microscopic morphology. This is in agreement with the hypothesis that the intrinsic quality of the oocyte and conditions during IVM largely determine the proportion of oocytes that develop to the blastocyst stage, while conditions during IVC are more important in determining embryo quality (Longergan, et al., 2006; Merton, et al., 2003). The possible influence of CODA air filtration during IVM and IVF remain to be investigated.

To identify the basis for the improvement in pregnancy rate in the present study, more fundamental research is needed to identify underlying mechanisms, since at the present time, little is known about preimplantation toxicology. Based on analysis of ambient air, Cohen et al., 1997, demonstrated dynamic interactive processes among air handling systems, spaces, tools, disposable materials and all items unique to their IVF laboratory. Concentrations of substances found can be considerably different from those of adjacent spaces and may

demonstrate an interaction between water-soluble and lipid-soluble solid phases such as those in incubators. For instance, culture media and mineral oil may act as a sink for different components. Even co-culture may operate by removing water-soluble traces from the immediate environment of embryos (Wiemer, et al., 1996).

Not all studies have demonstrated an improvement embryo quality and/or pregnancy rates when embryos were cultured in incubators with specialized filtration units. Battaglia et al., 2001 did not observe any change in embryo quality or pregnancy rate involving human embryos cultured in CODA-equipped versus non-CODA incubators. It should be noted that the degree to which laboratory air and incubatory gas atmosphere affect embryo development may depend on many factors unique to a particular laboratory and to the specific location of the laboratory. The two programs cited in which pregnancy rates were actually improved by the use of a CODA system in the incubator were located in Norfolk, Virginia and Campinas, Brazil, whereas the program in which no difference was noted was located in Seattle, Washington. The present study, involving cattle embryos, was conducted in a suburban area of The Netherlands. It is not possible in the present study to separate the possible contributions of contaminants from the compressed CO₂ and N₂ from the possible effect of atmospheric air that was also part of the incubator gas mixture. It is quite possible that the effectiveness of laboratory air and incubator purification systems is entirely dependent on unique and specific characteristics of each laboratory and the surrounding area.

In conclusion, our results show that, although many of the environmental conditions for gametes and embryos in IVP systems have been relatively standardised, there is still room to improve the overall outcome of an IVP system. We clearly demonstrated that the use of an intra-incubator carbon-activated air filtration system (CODA) during IVC of bovine embryos improved pregnancy rates following transfer of these embryos into recipient cattle.

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Fig 1 Effect of CODA intra-incubator gas purification on the distribution of different grades (IETS) of day-7 IVP embryos. Key: Grade 1 – excellent to good morphological quality, Grade 2 – fair morphological quality, Grade 3 – poor morphological quality.

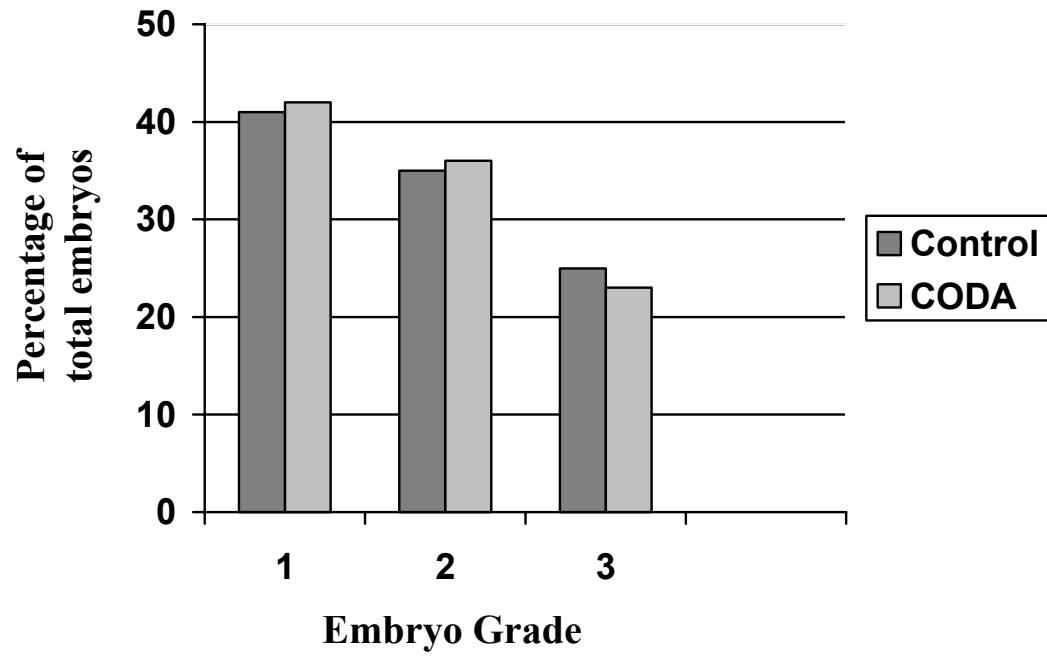


Fig 2 Effect of CODA intra-incubator gas purification on the distribution of different stages (IETS) of day-7 IVP embryos. Key: M – morula, EB – early blastocyst, B – blastocyst, XB – expanded blastocyst, HB – hatched blastocyst.

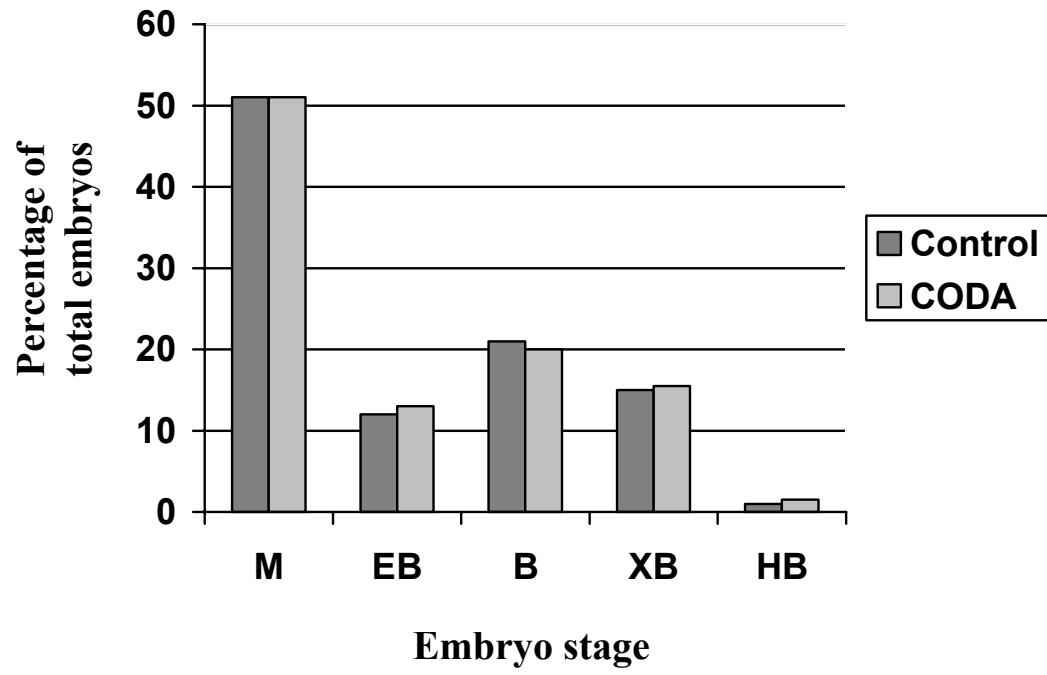


Table 1. Effect of CODA filter on IVP of embryos

Group	OPU sessions n	Oocytes in	Cleaved	Morulae/blastocysts		Blastocysts	
		IVC n	Day 4 n (%)	Day 7 n (%)		Day 8 n (%)	
Control	829	6566	3825 (58.3)	1536 (23.4)	1267 (19.3)		
CODA	778	6533	3901 (59.7)	1615 (24.7)	1326 (20.3)		

Table 2. Effect of CODA air purification during IVC on the pregnancy rate of both fresh and frozen/thawed IVP bovine embryos

Embryo	Incubator status	No. embryos	% Pregnant
Fresh	Control	401	41.0 ^a
Fresh	CODA	381	46.3 ^b
Frozen/thawed	Control	298	35.6 ^c
Frozen/thawed	CODA	337	40.8 ^a

^{abc}Means with different superscripts differ (P<0.05)