포스타 발표

P-1

돼지 난포란의 채란방법과 체외수정란의 체외발달에 미치는 요인

박성원, 홍승표*, 진종인, 정장용, 박희성
진주산업대학교 동물자원학과

본 연구는 돼지의 체외수정에 있어서 난포란의 채란방법, 체외수정 및 체외발달율을 조사하기 위하여 실시하였으며, 결과를 요약을 하면 다음과 같다. 도축장으로부터 수집한 난소의 난포에서 흡입, 세절 및 흡입후 세절법으로 채란을 실시하여 난소 1개당 각각 30.7, 50.8 및 103.5개를 각각 회수하여 회수율은 흡입후 세절법이 유의적(P<0.05)으로 높았다. 체외수정에 있어서 액상정액과 정소상체미부정액간의 수정율은 83.0 및 83.1%로써 이들간에 유의적(P<0.05)인 차이가 없었으며, 난할율은 각각 60.8 및 69.0%였다. 체외수정에 있어서 수정용 B.O와 TALP 배양을 각각 사용하였을 때 상실배로의 발달율은 12.6 및 17.7%로써 이들간에 유의적(P<0.05)인 차이가 없었으며, TCM-199와 NCSU-23 간의 배반포기로의 발달율은 TCM-199에서는 발달이 되지 않았으나 NCSU-23에서는 2.3%가 배반포기로 발달하였다. 소난관상피세포의 공배양을 실시하였을 때 배반포기로의 발달은 되지않았으나, 돼지난관상피세포와의 공배양에서는 2.5%의 발달율을 보였다. 체외수정이 이루어진 체외수정란에 β-Mercaptoethanol을 50, 100, 200 및 250 μM을 각각 첨가하여 체외배양을 실시하였을 때 상실배 이상으로의 발달율은 각각 22.5, 13.5, 19.0 및 22.0%로써 대조구의 16.6%와 유의적(P<0.05)인 차이가 없었다.

P-2

체외생산한 소 배반포 동결융해란의 포배강내 아미노산 분석

박용습*, 정연길, 이호준, 최은주, 김용엽[1], 정혜영[1], 노상호, 윤종택
한경대학교 동물생명자원학과, [1]공주대학교

본 연구는 동결·융해한 배반포의 포배강 내 아미노산 농도를 측정하여 포배강내의 아미노산 이용도를 알아보기 위하여 실시되었다.

도축된 한우 난소로부터 흡입, 채취한 난자를 선별하여 TCM-199에 10% FBS를 첨가한 배지에서 24시간동안 체외성숙시킨 후 30-42시간 동안 수정용 TALP 배양액을 이용, 체외수정하였으며 수정 후 난자는 4-well dish에서 난관상피세포와 공배양하거나 6-10개씩 30㎕의 배양액(배양용 TALP) 미소적에 분주하여 7-10일간 배양하였다. 배양 후 확장배반포로 발육한 수정란을 선별하여 10% glycerol 동결배지를 사용, -5℃에서 식빙한 후 완만동결법으로 동결하였다(-0.5℃/min로 냉각하여 -30℃). 융해는 0.3M의 sucrose가 함유된 PBS에 6%, 3%, 0% glycerol을 만들어 각각 5분간 정치하여 다단계 융해하였다. 아미노산 분석은 Biochrom 20(Phamacia Biotech, England)을 사용하여 실시하였다. 공배양 체계하에서 생산된 배반포를 동결-융해한 후 1% NEAA + 2% EAA 첨가한 mTALP 배양액 내에서 12시간 배양, 재확장 여부로 생존성을 판정한 후 확장된 배반포 만을 선별하여 포배강 내 아미노산농도측정에 공여하였다. 아미노산농도측정을 위해 BSA를 PVA로 대체한 TALP 배양액으로 20㎕의 미소적을 만들고 각각의 미소적에 배양액과 15~30개의 난자를 10㎕ 첨가하여 최종 volume을 30㎕로 조정한 후, 미세조작기를 이용하여 포배강 내의 액을 추출하였다.

공배양 및 단순배양체계에서 생산된 체외수정란의 동결·융해 후 생존성은 각각 52%(39/75) 및 21.5%(17/79)로 나타나 공배양유래 배반포가 유의적으로 높게 나타났다. 동결융해한 포배강 내 아미노산의 농도는 필수아미노산은 tyrosine(41.4pM/embryo), phenylala-nine, cysteine, threonine 등 10종이 검출되었으며, 비필수 아미노산은 glycine(40.8pM/embryo), serine, asparagine, alanine의 4종이 검출되었다. 본 실험의 결과로 보아 단순배양체계 유래의 수정란보다 공배양 유래 수정란의 동결성이 좋은 것으로 사료되며, 동결·융해 후 수정란의 확장 및 생존에 아미노산과 관련된 대사활성이 있음이 시사된다.

P-3

Production of Pups Following Artificial Insemination by Intrauterine Inseminator

Kong, I.K.*, S.G. Cho, Y.T. Im and S.I. Lee

Department of Animal Science and Technology, Sunchon National University

Artificial insemination (AI) with frozen spermatozoa involves many factors that may be critical and require careful attentions. These factors are semen collection, dilution, extender, equilibration, freezing, storage, thawing, time of insemination and deposit place of semen. This study was conducted to develop the intrauterine inseminator (IUI) to deposit of frozen semen into intrauterine instead of vagina and to evaluate the results obtained after AI by IUI. Two japanese spitzs (2 to 4 years of age) were used as semen donors. Semen was collected by manual masturbation into sterile glass collection tubes and separated into 3 fractions with only the sperm-rich fractions retained for further examination. Sperm motility >70%, sperm concentration of 200 to 400×106 cells/㎖, and up to 15% abnormal and dead spermatozoa were used. Each ejaculate was centrifuged at 400×g for 5 min and poured out the suspended solution, and then diluted with 2 ㎖Tris-buffer which was consisted of 2.4 g Tris, 1.4 g citric acid, 0.8 g glucose, 0.1 ㎍/㎖ streptomycin, 100 IU/㎖ penicillin, 20 ㎖ egg yolk to 100 ㎖ mili-Q water (Ext I) or supplemented with 7 ㎖ glycerol and 1 ㎖ Eques STM paste to 100 ㎖ (Ext II). The diluted semen was cooled to 5℃ in cold room, where the temperature in the sample reached 5℃. Two hour after beginning the cooling procedure, 2 ㎖ of Ext II, also at 5℃, was added and mixed by gently reversing the tubes several times during 1 h. The final sperm concentration for freezing was approximately 50×106 cells/㎖. After equilibration, the semen was loaded into 0.5 ㎖ straw and frozen on the liquid nitrogen vapour. The straws were thawed at 70℃ for precisely 6 sec. All the females were inseminated twice with 1 ㎖ of 25×106 cells/㎖ concentration at ovulation days estimated by ultrasonography. The semen was deposited into intrauterine by IUI. The catheter of IUI can passed the cervix and deposited frozen semen in intrauterine instead of vagina. After thawing of each straw, the frozen semen can survived over 70% motility. Four out of five females inseminated were pregnancy and three out of four pregnancy female were delivered 11 pups so far.

The results obtained indicated that Tris buffer could be frozen the canine semen successfully, and IUI can also be improved the pregnancy rate and reduced the semen concentration per AI rather than vagina insemination.

P-4

Expression of Green Fluorescent Protein Gene and Sexing in Bovine Embryos

T. Y. Kang[1], G. J. Rhol, Y. J. Chae[2], H. Lee[2] , S. Y. Choe[1] and H. J. Lee[1]
[1]*College of Veterinary Medicine, Gyeongsang National University,*
[2] *College of Veterinary Medicine, Seoul National University*

The efficiency of transgenic livestock production could be improved by early selection of transgene-integration and sexing of embryos at preimplantation stages before transferring them into recipients. We examined the possibility of green fluorescent protein(GFP) gene as a non-invasive marker for the early screening of transgenic embryos, and the simultaneous confirmation of the transgene and sex by multilpex PCR analysis.

The GFP gene(Takada, et al. Nature Biotechnology 15:458-461, 1997) was microinjected into the male pronuclei of bovine zygotes produced in vitro. The injected zygotes were co-cultured in TCM-199 containing 10% FCS with bovine oviductal epithelial cells in a 5% CO2 incubator. Seventeen(13.0%) out of 136 gene-injected bovine zygotes developed to blastocysts. The presence of injected DNA and their sex were simultaneously detected by multiplex PCR analysis and the expression of GFP was detected by observing green fluorescence in embryos under a fluorescent microscope. Eight(67%) of 12 embryos at 2-cell to blastocyst stage were positive in the PCR analysis, but only two(11.8%) of 17 blastocysts expressed the GFP gene. Their sex was determined as 7 female and 5 male embryos by the PCR analysis.

The results indicate that the screening of GFP gene and sex in bovine embryos by PCR analysis and fluorescence detection could be a promisible method for the preselection of transgenic embryos.

This experiment was supported by the Ministry of Agriculture (1995~1998), Republic of Korea.

P-5

Expression of Green Fluorescent Protein Gene in Rabbit Embryos

T. Y. Kang[1], X. J. Yin[1], Y. J. Chae[2], H. Lee[2] and H. J. Lee[1]

[1]*College of Veterinary Medicine, Gyeongsang National University,*
[2]*College of Veterinary Medicine, Seoul National University*

The efficiency of transgenic livestock production may be improved by early selection of transgenic preimplantation embryos. To examine the possibility of green fluorescent protein(GFP) gene as a non-invasive marker for the early screening of transgenic embryos, the GFP gene was microinjected into male pronuclei of rabbit zygotes. The injected zygotes were co-cultured in TCM-199 containing 10% FCS with rabbit oviductal epithelial cells in a 5% CO_2 incubator. The presence of injected DNA was detected by a PCR analysis and the expression of GFP was detected by observing green fluorescence in embryos under a fluorescent microscope. Seventy three (67.6%) out of 108 GFP gene-injected rabbit zygotes developed to blastocysts, among them 33 (45.2%) were fluorescence-positive. When the fluorescence-positive blastocysts were analyzed for the presence of GFP gene by PCR, 6 (54.5%) out of 11 blastocysts were positive, and all of the 8 fluorescence-negative blastocysts were also negative.

The results indicate that the screening of transgene in rabbit embryos by PCR analysis and GFP detection could be a promisible method for the preselection of transgenic embryos.

This experiment was supported by the Ministry of Agriculture (1995~1998) and KOSEF (961-0606-052-2), Republic of Korea.

P-6

Calcium Current Following Vitrification of Mouse Occytes

D. W. Kang*, J. H. Han and S. G. Hong
Department of Physiology, College of Medicine, Gyeongsang National University

There are many reports suggesting that Ca^{2+} influx and intracellular Ca^{2+} concentration ($[Ca^{2+}]$ i) are related to cell signalling in various cells and calcium ion plays the important role as a maturation factor in mouse oocytes.

Cryopreservation is a common used method for an efficient utilization of oocytes and embryos.

In this experiment, calcium current following vitrification was examined in order to improve effect of cryopreservation. Cryopreserved oocytes were incubated for 4hr after thawing and then used in the experiment. ethyleneglycol-ficoll-sucrose (EFS) was used as a cryoprotectant solution for vitrification.

The properties of the calcium current were investigated by the whole cell voltage clamp technique in zona removed fresh and cryopreserved metaphase II oocytes, which were collected from the oviduct of superovulation-induced mouse with PMSG and hCG treatment. The membrane potential was held at -80 mV and step depolarization was applied from -50 mV to 50 mV with increasing by 10 mv for 300 ms.

The results obtained were as follows

Current-voltage relationship showed that currents began to appear at -50 mV and reached maximum at - 10 mV. Ca^{2+} current in the mouse II oocytes was fully activated within 12 ms from the onset of step pulse and recovered to the steady-state within 50 ms with transient time course. Inward currents in cryopreserved oocytes were proved to be Ca^{2+} currents (iCa) like those in fresh oocytes. Current-Voltage relation in the fresh oocytes were similar with that of the cryopreserved oocytes

From the above results, properties of ionic currents elicited by voltage pulse were not changed in the fresh and cryopreseved metaphase II oocytes of mouse. These results suggest that cryopreservation does not critical impact on the calcium current of mouse oocytes under the present condition.

P-7

Roles of Carbohydrates and Amino Acids on Nuclear Maturation and *In Vitro* Fertilization of Bovine Follicular Oocytes Cultured in a Chemically Defined, Protein- and Hormone-Free Medium

W.S. Hwang[1*], H.M. Chung[2], S.E. Park[2], H. Sim[2], J.J. Ko[2], E.S. Lee[3], B.C. Lee[1], J.I. Park[1], S. Roh[4], Y.H. Choi[1], G.J. Ahn[1] and J.M. Lim[2]

[1]College of Veterinary Medicine, Seoul National University, [2]College of Medicine, Pochon CHA University, [3]Dept. of Veterinary Medicine, Kwangwon National University, [4]Dept. of Animal Life Resources, Hankyong National University

This study was conducted to examine how exogenous carbohydrates (glucose; Glu, lactate; Lac, pyruvate; Pyr) and amino acids (aa) affect nuclear maturation and in vitro fertilization of bovine follicular oocytes. Cumulus oocyte complexes (COCs) were collected from the ovaries of Holstein cows or heifers at a local slaughterhouse. Collected COCs were cultured in a chemically defined medium (modified bovine embryo culture medium; mBECM) with no supplementation of amino acid, carbohydrate, hormone and protein, and the tested substances with various combinations were added to the medium before maturation culture. At 22 to 24 hours after culture at 39 (C, 5% CO_2 in air atmosphere, COCs were inseminated in vitro by the standard protocol of our laboratories. Nuclear status of oocytes and fertilization of inseminated oocytes were evaluated at 24 hours after maturation culture and at 24 hours post-insemination, respectively. A generalized linear model program with ANOVA and a least square method in SAS was used for statistical analysis of the obtained data. In Experiment 1, COCs were cultured in mBECM, to which 1) no addition, 2) Glu (5.6 mM), 3) Lac (10 mM), 4) Pyr (0.5 mM) or 5) Glu+Lac+Pyr was added. More ($P<0.05$) oocytes reached to the metaphase-II (M-II) stage after the addition of Glu or Glu+Lac+Pyr than after no addition or the addition of Lac or Pyr (67 to 74% vs. 0 to 38%). The highest proportion of oocytes developed to the M-II stage after the addition of Glu and no oocytes could

develop to the M-II stage in carbohydrate-free mBECM. Regardless of the type of carbohydrate supplementation, germinal vesicle breakdown was observed in all treatment groups, but more oocytes arrested at the germinal vesicle stage in carbohydrate-free medium (34%) compared with in any of carbohydrate-containing media (0 to 2%). In Experiments 2 and 3, COCs were cultured in mBECM supplemented with Glu and 0, 0.01, 0.1, 1, 5 or 10% of BME essential and nonessential aa solutions was added to the medium from the onset of maturation culture. Compared with no addition, the addition of 0.01, 0.1 or 1% aa did not promote nuclear maturation (71 to 74%) and the addition of 5 or 10% aa significantly inhibited the maturation process (0 to 52%). Total fertilization rate of oocytes after in vitro insemination did not significantly differ among 0 to 1% aa concentrations (89 to 91%). However, the formation of both male and female pronuclei in fertilized oocytes was higher in oocytes matured in 1% aa-containing medium (56%) than in oocytes matured in 0 or 0.01% aa-containing medium (33 to 35%). Proportion of polyspermic oocytes was not affected by the addition of 0 to 1% of aa. In conclusion, the results of these experiments demonstrated that glucose and essential and nonessential aa were important for nuclear maturation and pronuclear formation of bovine follicular oocytes. However, they may exert different supporting role in oocyte maturation process.

P-8

Efect of antioxidants on development of *In Vitro* matured and fertilized Korean native cattle embryos

S.J. Moon, J.H. Kim, K.H. Myung, S.S. Sun, E.K. Kim*
Department of Animal Science. Chonnam National University

The objective of this study was to determine effects of α-tocopherol and L-ascorbic acid on the development of korean native cattle fertilized oocytes obtained from *in vitro* matured and fertilized oocytes. The follicular oocytes were cultured in TCM-199 medium containing PMSG (10 IU/㎖), HCG (10 IU/㎖), and 10 % FCS for 24 hrs in incubator with 5 % CO_2 in air at 38.5℃. The cleavage rate of fertilized oocytes in CR_1aa and mSOF containing 0, 2.5, 5.0, 7.5 μM of α-tocopherol and 0, 50, 62.5, 75 μM of L-ascorbic were 67.3 %~75.3%, 61.8%~69.7%, 82.7%~93.5% and 79.5%~87.1%, respectively. However there were no significantly differences between groups. When the fertilized oocytes were cultured for 7 days in CR_1aa and mSOF with α-tocopherol, the rates of development to morulae and blastocyst were significantly higher than control group. When the fertilized oocytes were cultured for 7 days in CR_1aa and mSOF with L-ascorbic acid, the rates of development to blastocyst were 0~9.3% (CR_1aa). And the rates of development to morulae were 22.0~44.3% (mSOF). The morulae and blastocyst rate of control group were signigicantly lower than that of oocytes cultured with L-ascorbic acid ($P<0.05$). Addition of 2.5 μM α-tocopherol and 50 μM of L-ascorbic acid to the culture medium increase the incidence of embryos developed to the morulae and blastocyst.

These results suggested that the addition of α-tocopherol and L-ascorbic acid enhanced development to the morulae and blastocysts of *in vitro* derived fertilized oocytes.

P-9

OPU유래 유전자 미세주입 체외수정란의 생산

최선호, 류일선, 김일화, 박수봉, 연성흠, 진현주,
서상욱, 손동수, 한용만*, 이경광*
*농촌진흥청 축산기술연구소, *생명공학연구소*

고능력을 지닌 젖소로부터 OPU유래 수정란의 생산은 조기에 유전적인 개량을 향상시킬 수 있다. 본 연구는 OPU에 의해 채취된 난구복합체(COCs)를 체외성숙·수정을 통하여 유전자가 미세주입된 수정란이식이 가능한 배반포기 수정란의 생산을 목적으로 실시하였다.

COCs는 4~5세인 7두의 Holstein암소로부터 주 1회 채취하였다. OPU는 hand pump(10psi 이하, Nalgene) 흡입방법으로 실시하였고, 250㎎/ℓ heparin이 함유된 D-PBS로 채취된 COCs를 침지하여 혈액 응고를 방지하여 COCs의 관찰을 용이하게 하였다. 채취된 COCs는 1㎍/㎖ estradiol-17β와 25 ㎎/㎖ gentamycin이 함유된 50㎕ 소적의 TCM199배양액에 38.5℃, 5% CO_2 95% 공기인 배양기에서 22시간 체외성숙을 실시하였다. 체외수정은 동결정액을 percoll 처리에 의해 세정하고, SP-TALP(Parrish, 1988)로 체외수정능을 획득시킨 후, 정자 농도를 2×10^6sperm/㎖로 조정하고 Fert-TALP(Yanagimachi, 1988)로 20시간 체외수정을 실시하였다. 체외수정 후 난구세포를 TL-HEPES에서 피펫팅에 의해 제거하고 15,000rpm으로 7분간 원심분리를 실시하여 전핵이 관찰될 수 있도록 하였다. 미세주입을 위한 DNA는 사람 성장인자(2.1 kbp)를 소의 베타 카제인 promotor와 결합하였으며, 0.1mM EDTA가 함유된 10mM Tris-HCl로 4ng/㎕로 희석하여 주입하였다. 미세주입이 성공한 수정란은 3㎎/㎖ BSA가 함유된 CR1aa배양액으로 48시간 배발생을 유도하였고, 이어서 mouse embryonic fibroblast monolayer로 5일간 공배양을 실시하였다. 미세주입 체외수정란은 수정 후 7~8일에 관찰을 실시하였다.

OPU에 의해 채취된 COCs는 각 개체에 따라 현저한 차이를 보였으나, 형태적으로 사용 가능 COCs는 체외성숙 및 체외수정을 유도할 수 있었다. 미세주입 체외수정란의 배발달율은 상당히 큰 차이를 보였다(0~62.5%). 또한 4개의

유전자 미세주입 배반포기 수정란을 생산하였다.

이상의 결과로부터 간단 OPU채취법에 의해서도 이식 가능 수정란의 생산이 가능함을 시사하며, 호르몬을 이용한 난포형성 촉진과 주기적인 COCs채취에 의해 더욱 향상시킬 수 있을 것으로 기대된다.

Table 1. The production of microinjected blastocysts derived by OPU

Cows	No. of trial	No. of IVM	No. of IVF	No. of embryos microinjected	No. of embryos cleaved	Cleavage rate (Mean, %)	No. of blastocyst
1	4	23	23	8	5	62.5	1
2	9	79	75	32	17	51.7	2
3	3	19	19	6	2	22.2	0
4	1	3	3	0	0	0	0
5	2	3	2	2	0	0	0
6	2	6	6	5	2	41.6	1
7	1	9	9	2	1	50.0	0

P-10

한우 체외 수정란이식에 의한 쌍자 송아지 생산

김 의 성

현대가축인공수정소, 전남 무안군 무안읍 성내리 114-1

수정란이식 기술은 가축개량과 생명공학기술의 응용에 있어서 지대한 공헌을 하였다. 또한 저능력우나 배란장애에 의한 불임 등의 치료에도 이용될 수 있는 효용성이 높은 기술로 인정되어 왔다. 따라서 본 시험은 한우 체외수정란을, 능력이 떨어지거나, 공태기간이 길어 젖소로서 능력이 떨어지는 경산우를 대상으로 수정란이식을 통한 쌍자생산을 목적으로 1997년과 1998년에 걸쳐 실시하였다.

수란우는 전남·광주 일원의 1산 이상의 자궁상태가 양호하고, 조사료를 많이 급이한 경산우를 선발하여 발정발견 후 평균 25시간에 한우 및 젖소 동결정액으로 수정을 실시하였으며, 인공수정 후 7.5~8일째에 수란우가 한우의 경우는 1개, 젖소의 경우 2개, 수정란이식을 실시하였다. 또한 별도의 수란우의 인위적인 발정유기는 시도하지 않고, 자연발정이 관찰된 것만을 수란우로 이용하였다. 본 시험에 공시된 한우 체외수정란은 전라남도축산기술연구소 축산시험장에서 생산된 신선란 및 동결란을 이용하였다. 신선란의 경우는 공급지로부터 현장까지 30분 이내의 거리인 경우에 실시하여 수정란 이송시 온도변화에 대한 충격을 최소화하였다.

이식결과로 1997년도에는 한우 동결란만으로 이식하여, 수태율은 91.7%(110/120)이었고, 이중 단자는 42두(38.2%), 쌍자는 68두(61.8%)를 분만하였다. 1998년도에는 수란우를 한우와 젖소를 대상으로 이식하였으며, 신선란의 경우 84.3%(43/51)의 수태율을, 동결란의 경우 61.9%(39/63)의 수태율을 보였다. 분만결과는 신선란의 경우 쌍자 및 3자가 36두로 83.7%(3자: 21두)였으며, 동결란의 경우 28두로 71.6%를 나타냈다. 1998년도에 동결란의 수태율이 1997년도에 비해 떨어진 것은 실험보조자의 교체로 인한 숙련도의 결함에 의한 것으로 여겨진다.

이상의 결과로 미루어, 수란우의 적절한 사양관리 및 철저한 번식관리, 그리고 적절한 수정란의 처리에 의해 쌍자 혹은 3자의 생산도 가능하여, 이러한 기술을 이용하여 번식효율 증진을 꾀할 수 있을 것으로 여겨진다.

P-11

1998년 국내 소 수정란이식 현황

김일화, 손동수
농촌진흥청 축산기술연구소

1998년 국내 수정란이식 현황을 파악하기 위하여 전국의 수의·축산분야 대학, 국·공립 연구소, 농업기술센타, 축협, 동물병원 및 가축인공수정소 등의 382개 관련기관에 대하여 1998년 1월 1일부터 12월 31일까지 1년동안 실시한 소 수정란 생산, 이식 및 임신진단 결과를 설문을 통하여 조사하였다. 수정란 이식 현황을 제출한 36개 기관의 자료를 분석한 결과는 다음과 같다. 체내수정란을 생산하는 기관은 국립기관 2개소, 대학 1개소, 지방자치단체의 축산관련기관 6개소, 축협 및 민간시술소 4개소 등의 13개소 였으며, 체외수정란을 생산하는 기관은 국립기관 2개소, 대학 4개소, 지방자치단체의 축산관련기관 8개소, 축협 1개소 등의 15개소 였다. 수정란이식을 실시한 기관은 국·공립 연구기관 4개소, 대학 4개소, 농업기술센타 20개소, 축협 및 민간시술소 7개소 였다.

체내수정란의 생산은 한우 225두 및 젖소 91두를 과배란처리하여 한우177두와 젖소 75두에서 각각 964개(5.4개/공란우)와 296개(3.9개/공란우)의 이식가능 수정란을 회수하였다. 체외수정란의 생산은 초음파난자채취기를 이용하여 소의 난소에서 채취한 난포란으로부터 167개, 도축된 소의 난소에서 채취한 난포란으로부터 21,688개의 수정란을 생산하였다.

수정란이식은 한우 수정란이 3,404두, 젖소 수정란이 757두에 이식되어 모두 4,161두가 이식되었다. 수정란의 상태별로 구분하면 체내수정란은 982두(23.6%), 체외수정란은 3,179두(76.4%)에 이식되었으며, 신선수정란은 27.1%(1,129두), 동결수정란은 72.9%(3,032두)를 차지하였다. 수정란의 이식 형태별로는 수정란 1개 이식이 30.2%(1,257개), 수정란 2개 이식이 15.2%(632개), 인공수정후 수정란 추가이식이 54.6%(2,272개)를 나타내었다.

임신진단이 실시된 2,854두에 대한 수태율 현황은 체내수정란이식에서 신선수정란 1개 이식 53.3%, 2개 이식 40.5%였으며, 동결수정란 1개 이식 38.7%, 2개 이식 33.3%였다. 체외수정란이식 수태율은 신선수정란 1개 이식 39.5%, 2개 이식 50.9%이었으며, 동결수정란 1개 이식 23.4%, 2개 이식 26.1%를 나타내었다. 따라서 신선수정란이 동결수정란보다, 체내수정란이 체외수정란 보다 높은 수태율을 나타내었다.

The 1997 Embryo Transfer Statistics from Around the World

A Data Retrieval Committee Report
M. Thibier, Chairman of the IETS Data Retrieval C

Summary

For the 7th consecutive year, the IETS Data Retrieval Committee has completed survey of the ET industry. It held its annual meeting in Boston, January 1998. At this new tables for collecting embryo data from additional species as well as *in vitro* produced were agreed upon. These tables were distributed, at that time, to the local or regional ET collectors. The results of the network for collecting this data are reported here.

The total number of *in vivo* derived embryos from cattle has slightly decreased (b mainly due to fewer embryos transferred in Asia, North, and South America. By contra continents, such as Europe, have increased their number of embryo transfers. In total, 36 *vivo* derived embryos have been transferred worldwide. In contrast to this slight reduction was a dramatic increase in the number of *in vitro* produced (IVP) embryos that were e transferred. This is the key event of the year! In vitro produced embryos have reached th and in some countries are used on a routine basis by the farmers. Over 30,000 IVP cattle have been transferred. Therefore adding up the *in vivo* and the *in vitro* data, < B>391, embryos have been transferred worldwide. Like last years data, embryo transfers from species have been reported. The species include ovine, caprine, equine, swine and cervid IETS Data Retrieval Committee was happy to see that this network was continuing to members. This was illustrated by numerous queries for statistics from Universities International governing bodies. It was also encouraging to see that new countries, s Paraguay, have been inclu ded in the network. The Chairman once again thanks very m those, from close to 50 countries throughout the world, that helped collect this data.

Introduction

The IETS Data Retrieval Committee has worked very efficiently this past year to colle statistics from many countries. The IETS wants to thank its members who dedicated some time to that effort.

ObjectivesandMethodsoftheCommittee

The Committee held its meeting at the IETS yearly conference in Boston, in January

1998, and discussed, at large, its objectives and the most appropriate methods to achieve those goals. It was clearly established that the present system, through local or regional collectors of data from all teams involved in ET in a given area (see Thibier, 1992, IETS Newsletter, 10 (4): 11), is the best one. This is provided that the collectors convince their citizens and colleagues of the benefits the whole ET industry can receive from those world reports. Further, practitioners must be willing to release the statistics from their yearly operation. It is rewarding to see that this is most often the case, but there are places where these requests for inform ation are still met with some reluctance. It has been re-conveyed that this data is kept strictly confidential by the local or regional collector, as had always been observed.

Continued thanks to the professionalism of the collectors who never use this p information for their own benefit. The system works fine even though some improvements made by getting still more countries in the system. Particularly important are the countrie former USSR and India where we have had difficulty t he past two years reaching a natio set of data. It is undoubtedly a more difficult job in some areas of the world than However, all IETS members should be able to help in this regard. Members can either where relevant, somebo dy who has the authority and confidence of its country people or and communicate the need for a good indication of our world ET industry activity.

On the other hand, the Committee is happy to see new countries joining this netwo Paraguay, and others returning, such as Mexico. Other points of improvement discussed in refer to additional species that we want to cover and also on the renovated format of the putting the figures together. These forms will be sent to the l ocal or regional collectors, and sent back to the Chair of the Committee for final computerization.

On those forms, it was decided to stop requesting the breed distribution and just emb cattle. Even though simplified, this year exper ience has shown that most often breeds w reported and therefore makes the use of that data impossible.

Major entry of *in vitro* produced embryos in routine field operations in cattle.

In cattle, we are now collecting data from both *in vivo* derived and *in vitro* produce The major observation one can make from this year data is that *in vitro* produced embr their place in the routine field operati ons in some countries.

Bovine *in vivo* derived embryo transfers

The *in vivo* derived embryo data presented in Table 1, show a slight reduction (10-1 total number of embryo collections as well as in the total number of embryos transferred were a little more than 350,000 embryos reported to have been transferred this year. This r originates from several parts of the world, North America, South America, and Asia.

This is however, in contrast with what was observed in Europe. In Europe, there w significant increase in the numbers of transfers (> 10%) compared with the previous year.

These slight changes do result in some re-distribution by region from that reported last

This year (1997), one can see that more than two-thirds of the embryos transferred

Europe (35.5%) and North America (34.6%). Asia and South America transferred approximately 14 and 10% of the total respectively, which is a little less than the previous year. Oceania reported more than 14,000 embryo transfers, thanks to a more efficient data collection network this year, while Africa remained stable. It is also interesting to observe in terms of fresh vs. frozen embryos that were transferred, 53% of the total were frozen. The percentage seen in Europe is exactly reversed in North America. It was claimed in previous years, that the embryos from dairy breeds are transferred as fresh more often than those from the beef breeds. This is because more beef embryos are carried over in time and perhaps in space than dairy embryos. The dairy breeders want to take advantage of the higher genetics from those embryos right away. By contrast the beef breeders more often try to position themselves in a domestic or international markets and to do this, need to have those embryos banked. It is not known at th is stage whether this still holds true here and if this would explain the opposite trend of the European and North American distribution of fresh vs. frozen embryos. We failed in trying to obtain the breed distribution of embryos transferred in a consistent manner worldwide, and therefore, we cannot comment on this hypothesis at this stage. In South America and even more in Asia, most embryos are transferred as frozen, while the majority of embryos in Africa and Oceania are transferred as fresh. It is also worthwhile to note from the bulk data, the number of transferable embryos per flush is over 5.5 (5.54 exactly) which shows quite a high efficiency in superovulation. This figure has slightly increased as compared to the previous year (5.4).

Although this report is not technical, it is interesting that some comments given by the show that direct transfer of frozen embryos in the bovine has increased dramaticall pregnancy rates with these embryos are ~58% in North America and close to this figure countries of Europe. It is also of interest to note that frozen direct transfer is overta classical "so-called" glycerol technique. Close to 60% of embryos transferred used the transfer procedure both in North Ame rica and Europe. As always it is difficult to get an idea of the international movements of embryos. The US however, reports that approxi 11,000 embryos were exported. Canada exported 8,531 *in vivo*-derived bovine embryo imported 350. The People Republic of China imported more than 1,000 bovine embryos from Canada.

Table 1. Overall Bovine Embryo Collection and Transfer Activity in 1997.

Continents	Flushes	Transferable Embryos	Number of Embryos Transferred			
			Fresh	Frozen	Total	%
Africa	3,031	17,452	5,238	3,528	8,766	2.4
N. America	27,681	178,818	65,570	59,383	124,953	34.6
S. America	5,380	24,425	18,542	16,712	35,254	9.8
Asia	14,435	72,466	11,416	38,308	49,724	13.8
Europe *	28,706	150,428	59,997	67,525	127,522	35.4
Oceania	3,074	12,669	7,610	6,827	14,437	4.0
Total	82,307	456,258	168,373	192,283	360,656	100.0

* The European data is derived from the statistics of AETE, 1998. Due to some typing those proceedings, some data has been corrected and the data of Finland and Turkey, not from the AETE, has been added.

From North America, the US reports a total of 76,028 transferred embryos of which 56 transferred as fresh embryos. Those figures are estimated to cover about 80% of the tota activity. From Canada, 60 operations responded total ing 10,947 flushes, 65,653 transf embryos, and 44,845 embryos transferred, 47.9% of these were transferred as fresh embryos Additionally, the Canadian report indicates that 71% of the donors flushed were of dai Mexico has flushed 390 fema les and more than 4,000 *in vivo* derived embryos ha transferred.

As far as Europe is concerned, Table 2 reports the numbers and trends from countries, most of them are increasing in number or are stable and those with some decli only a marginal decrease. One can see that 4 countries do transfer almost 20,000 emb more: France (with over 30,000), the Netherlands, the United Kingdom, and Germany.

Table 2. The Top Twelve European Countries Ranked According to Numbers of Bovine E Transferred in 1997 (AETE, 1998*).

Country	Number of Flushes **	Number of Embryos Transferred ***
France	6,814	31,297 ∅
Netherlands	5,530	23,607 ≅
United Kingdom	5,192	21,444 ∅
Germany	3,837	19,620 ≅
Belgium	1,821	6,741 ∅
Italy	1,122	9,563 ∅
Ireland	883	5,610 ∅
Czech Republic	790	4,159 ≅
Denmark	613	3,176 ∅
Spain	508	1,527 ∅
Switzerland	397	2,358 ∅
Sweden	382	1,884 ∅

∅ ≅ ∅ Evolution as compared to the previous year.

* Some of the countries have sent corrections since the AETE meeting due to typing the proceedings. They have been corrected accordingly.

**This corresponds to the number of collections of in vivo derived embryos.

***This corresponds to the total number of embryos transferred including those produced, when relevant, as to better illustrate the appropriate embryo transfer a

The five top countries outside North America and Europe and their relevant numbers in Table 3. Japan and Brazil remain the two countries with the highest numbers in this group.

However, their number of embryos transferred decreased by 30 and 50% res compared to last year. Some of this reduction might come from a couple of ET teams w not responded to the request, but it also indicates a slight slow down of the industry countries. By contrast, Argentina seems to have a very active ET industry as close to 2,00 were flushed with close to 10,000 transferable embryos collected. The vast majority embryos collected there were from the various beef breeds with only 23.5% of the embry dairy breeds. Approximately 20% of the frozen embryos transferred in Argentina were impo

Table 3. The Top Five Countries Outside Europe and North America (1997 data).

Country	No. Flushes	Number of Embryos Transferred		
		Fresh	Frozen	Total
Japan	13,231	8,433	31,946	40,379
Brazil	3,319	13,724	10,361	24,085
Argentina	1,855	4,142	5,135	9,277
South Africa	3,011	5,213	3,407	8,620
New Zealand	1,567	3,930	3,830	7,760

In Vitro Produced (IVP) Bovine Embryos Transferred

This is the second year we have published statistical data for in vitro produced bovin As eluded too earlier, one can see (Table 4) a dramatic increase in the number of suc produced embryos in cattle. More than 30,000 of such embryos have been transferred exactly). However, only New Zealand, Europe, Asia, particularly but not only Japan, have distinguished between *in vivo* and *in vitro* derived embryos. There are some IVP embryos produced and transferred elsewhere in the world, but as yet they are not clearly id Nevertheless, there is a lot to learn in terms of relevant strategies for use of this *in vitro* germplasm in cattle. The number of transferable embryos is close to 30,000 in Asia as from Japan, Korea, and Taiwan. This high number is related essentially to mass pro reported in Korea. This however, covers both experimental and commercial data, and it been possible to divide the proportions associated with commercial operations. In New Z more than 1,000 transferable embryos have been produced following 1,057 trans vagino collections with two thirds being (semi) commercial. In Europe, over 10,000 transferable *i* produced embryos have been reported from 10 countries: Belgium, Czech Rep., France, Hu

Ireland, Italy, Netherlands, Portugal, Spain, and United Kingdom. Most of them, and parti Italy and the Netherlands, have organized themselves very efficiently to sell IVF-produced e services to the farmers. This allows them to participate in the improvement of the genetic the cattle herds, mainly but not exclusively dairies. One can also see from the numbers transferred, there are more frozen embryos transferred than fresh embryos transferred. This holds true in Europe, Japan, and Korea, indicating that whatever the pregnancy rates reported from deep frozen IVF produced embryos, such embryos can be transferred on a commercial basis. It is of note that two countries in Europe are exception to this, Ireland and the Netherlands which both have the majority of their IVF produced embryos transferred as fresh (approximately 1,300 and 1,800 respectively).

Table 4. The Number of Transfers of Bovine *In Vitro* Produced Embryos in 1997 (AETE,

Country	Transferable Embryos Collected	Number of Embryos Transferred		
		Fresh	Frozen	Total
Asia	28,622 *	3,151	7,409	10,560
Europe	11,674	7,166	11,142	18,308
Oceania **	1,336	1,701		1,701
Total		12,018	18,551	30,569

* This is the sum of both experimental and commercial embryos.
** The country involved here is New Zealand.

Embryo Transfers in Other Species

As far as the other species are concerned, we have improved our system of collection get more species information reported. We have received reports on horses with some e being collected and transferred, for example Mexico reports 29 collections, 8 transf embryos, and 2 pregnancies. Iran reports some 29 transfers of fresh embryos fro Thoroughbred and Turkoman breeds although it was not clear whether this was done last over the last few years. Other countries quote some pregnancies underway in this species r from ET, but there are no figures given. Clearly this is one point where the Committe IETS members could try to make an effort in helping to collect this data. For other r some countries have a good indication of what has been done, particularly where there international movement allowing the collector to clearly identify the operators. Table 5 colla information and shows that the number of flushes and transferable em bryos collected h changed much in sheep as compared to last year. The number of embryos transferred howe decreased slightly due to a lower number in fresh embryos. By contrast, goat embryo generated a great deal of activity during 1997. Close to 10,000 goat embryos have been tra mainly in the Southern Hemisphere. France and Mexico have each reported that ~30

embryos have been transferred. Finally, cervids are once again identified as a genus, which technology. Both Australia and New Zealand report having transferred cervid embryos eithe or frozen (approximately 500 combined).

We have for the first time this year, thanks to our colleagues from Canada, a report on embryo transfer in swine, Elk and Bison. From 5 sows collected, 105 embryos were assessed as transferable. In Elk and Bison, 241 flushes were performed giving 1,088 transferable embryos out of which 893 were frozen. From those, 295 were transferred fresh (pregnancy rate 48% according to Mapletoft) and 419 frozen-thawed embryos were transferred with a pregnancy rate of 45% reported. There are almost 700 embryos from these species banked at this time in Canada. Some embryos were reported transferred in other species, for example, South American camel ids, not only in zoos. We hope to get more of that data together next year.

Table 5. Small Ruminant ET Activity in 1997.

Continents	Flushes	Transferable Embryos	Number of Embryos Transferred			
			Fresh	Frozen	Storage	Export
SHEEP						
South Africa	128	345	121	168	125	143
Mexico	25	150	120	20	10	
Canada	51	403	83	161	310	30
Australia	79	328	380	199		143
Total	283	1,226	704	548	345	316
GOAT						
South Africa	64	301	105	269	300	269
Mexico	35	175	130	120	15	
Australia	613	6,475	7,776	1,007		894
Argentina				142		
New Zealand	200	850				
Total	912	7,801	8,011	1,608	315	
CERVIDS						
New Zealand	88	495	395			
Australia	2		24	51		
Tota	90	495	419	51		

Conclusion

In conclusion, the ET industry in 1997, has had a very busy year with close to 400 embryos transferred. In vivo derived embryos are still in the majority, approximately 360,0 close to 30,000 in vitro produced emb ryos were also transferred. This last figure is the key event of this past year in our ET industry. We have shown the world that this technology can be used routinely to benefit farmers. Further, it can be used not only to transfer fresh but also deep fro zen-thawed embryos as illustrated in this article. As far as the IETS Data Retrieval Committee is concerned, we still have to improve our collecting network to encompass more countries, more teams in the countries, and probably more species to have an eve n more accurate set of data. The Committee will meet again at the next IETS Annual Meeting in Quebec City, and all members are welcome to make suggestions to improve our data collection even further.

It is the privilege of the chairman to gratefully acknowledge the most valuable help participated in this worldwide network of ET data retrieval and more particularly all of the G. Bo, R. Botha, H. Bong Seok, Jingbo Chen, R. Elaisch , M. Haynes, Y. Heyman, M. A A. Iritani, M. Kuran, R. Mapletoft, L. Marman, A. Peltomaki, A. Pugh, B. Purwantar Rodrigues, N. Schutte, Shan- Nan Lee, A. Shirazi, and B. Stroud.

(이 자료는 International Embryo Transfer Society에서 발행하는 *Embryo Tran Newsletter*의 Volume 16, Number 4, December 1998에서 발취한 내용임)