IN VITRO EMBRYO PRODUCTION
SOME CONSTRAINTS AND SOLUTIONS

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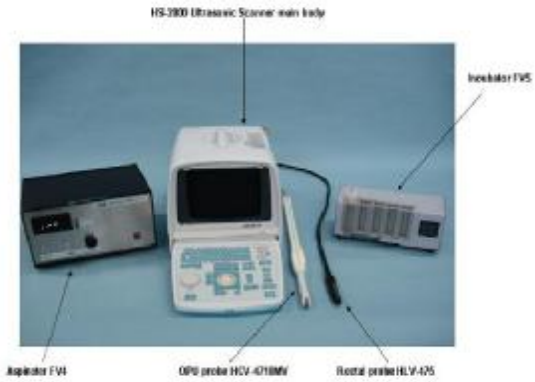
OOCYTE RESOURCE AND OVUM PICK-UP

Production of animal embryos in the laboratory has several advantages in comparison with conventional superovulation and embryo transfer (ET). These advantages can be listed as follows:

- *First*, *In vitro* Embryo Production (**IVEP**) can be used on problem bovines such as females that fail to respond to superovulation treatment.
- *Second*, IVEP can be used to salvage the genetic potential of terminally ill females that would not be expected to respond to conventional ET.
- *Third*, semen from different bulls can be used to fertilize oocytes harvested from a cow resulting in embryos with different sires being produced at the same time.
- And f*ourth*, oocytes for IVEP can be obtained from the ovaries of live donor using Ovum Pick-Up (**OPU**), or from the slaughter ovaries.

Due to low efficiency of superovulation and high cost of Follicle Stimulating Hormone (FSH), the *in vitro* embryo production technology has been researched in the last decade as an efficient alternative to *in vivo* system to produce embryos for faster propagation of elite germplasm as well as for research in the field of developmental biology and emerging biotechnologies. The application of the OPU for supplying oocyte to IVEP were increasing world-wide from 30,000 embryos transferred in 2000 to over 300,000 in 2009, a tenfold increase in one decade (IETS, 2011).

Constraints: in some countries like India where oocytes of cattle are not available as cows remain the holy animal and there is ban over slaughter of cow, or in Vietnam where bad oocytes of old/ill cows at slaughter house were not good for IVEP and that's why the technique like OPU is one of the alternatives to get developmentally competent oocytes. In Viet Nam, IVEP in dairy cattle get more difficult because of most of dairy cows were only eliminated by too old or problems of reproduction after many treatments with hormone, it means that the ovary was seriously influenced. So, oocytes from slaughter dairy cow ovaries were not high developmental competence. Evidence suggests that the intrinsic quality of the oocyte is the key factor determining the proportion of oocytes developing at the blastocyst stage. The oocyte maturation is a complicated process and the selection of oocytes for culture *in vitro* only by morphology, therefore is not enough to obtain better IVM and IVF. There is considerable evidence that the medium and conditions of oocyte culture also have impacts on the development of bovine embryos *in vitro*. These processes involve numerous levels of checks and balances, and are sensitive to regulation of endogenous and exogenous factors.



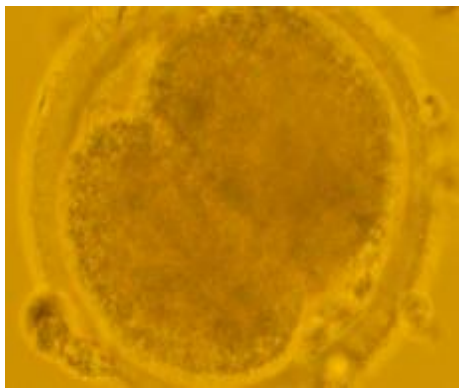
OPU Ultrasound system

Trying OPU ultrasound system on live cow

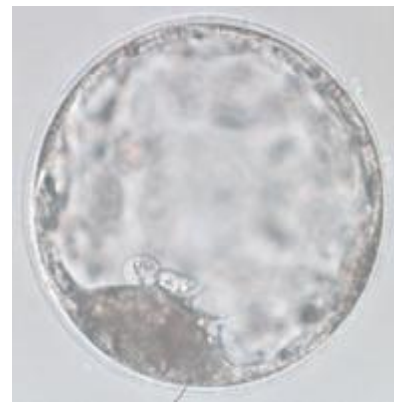
Solution: Transvaginal Ovum Pick-Up has been considered a good choice for oocyte supply to IVEP. Biotechnology Department (IAS) has been trying to apply OPU system, including ultrasound machine (HS-2000V, Honda Electronic Ltd.), special transvaginal probe (80-100mm) and 18G needle with needle guidance system for OPU, vacuum aspirator with suction powers between 90 and 100 mmHg, corresponding to an actual suction power of 36ml/mm of water, and incubator for warming (37-38^{o}C) recovered follicle fluid in Falcon tube (attached picture). Our initial result showed that OPU technique is rather difficult and complex, but we can conduct OPU technique in both laboratory and live cow. After two experiments, 2 – 5 oocytes recovered on ovary (34 oocytes recovered from 12 ovaries) and IVEP results were 91.17% mature oocytes (IVM), 32.25% zygotes (IVF) and 70% morula embryo (IVC). However, OPU on live cow is still rather difficult with some different unsuccessful reasons after 13 OPU trials.

BLASTOCYST RATE AND IMPROVING CULTURE MEDIA

In recent report of Fulvio and Cecilia (2010), as a consequence, in species where this technique is more developed, as in ruminants, on average, no more than 40% of the oocytes matured *in vitro* can develop to the blastocyst stage. At this point they can be transferred into a recipient animal where only 40 to 60% of the embryos goes to term. These values refer to cattle IVP embryos and to experienced laboratories. Other species, like pig or horse, typically show lower results.



Zygote with two cells



Blastocyst with ICM and blastocoel

Constraints: Cattle embryo growth are usually blocked at 4-8 cells (Kay Elder and Brian Dale, 2000) or 8-16 cells stage (Memili and First, 2000) with some reasons (metabolic factors, genetic factors, hormones, heat shock, apoptosis…) which prevent embryonic genome activation (EGA) leading to arrest embryo growth. Although, some other studies reported higher blastocyst rate (42%) of IVEP. In Northern Vietnam, general blastocyst rate of IVEP is still not high in cattle. Many researches have been conducted for improving blastocyst rate of IVEP by adding hormone (FSH/LH, hCG, eCG) into maturation media, growth factors (EGF, IGF, FGF) into maturation and culture media (SOF, KSOM, CR1aa) or co-culture with other cells (bovine oviduct epithelial cells, cummulus cells or trophoblastic vesicles, established cell line, buffalo rat liver cells…). Optimal culture medium is still a expectant solution.

Solutions: in our condition, we have conducted some experiments by adding IGF-1 into IVC medium at different levels. The initial results showed that:

- Bovine morula and blastocyst rate of IVC medium added 50ng/ml and 100ng/ml IGF-1 (Sigma) were 48.28% and 45.95%, respectively, comparing to 28,79% of the control (without IGF-1).
- Swine morula rate of IVC medium added 10ng/ml and 20ng/ml IGF-1 were 20.6% and 12,5%, respectively, comparing to 10.4% of the control (without IGF-1).

REFERENCES

Fulvio Gandolfi and Cecilia E. Gandolfi. 2010. Advantages and limitations of reproductive technologies applied to conservation programs. Recent Developments in Reproductive Technologies. "International Strategic Program for Conservation of Animal Genetic Resources for Food and Agriculture" sponsored by OECD Co-operative Research Programme.

International Embryo Transfer Society-IETS. 2011. Statistics and Data Retrieval Committee Report - The year 2010 worldwide statistics of embryo transfer in domestic farm animals - Prepared by Brad Stroud, DVM

Karadjole, M., Getz, I., Samardzija, M., Macesic, N., Matkovic, M., Marek, Z., Karadjole, T., Bacic, G., Dobranic, T. and Ppoletto, M. 2010. The developmental competence of bovine immature oocytes and quality of embryos derived from slaughterhouse ovaries or live donors by ovum pick up Vet. Archive 80, 445-454, 2010.

Kay Elder & Brian Dale 2000. In vitro fertilization. Second Edition. Cambridge University Press

Memili E, and First NL. 2000. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression compared with other species. Zygote 8:87-96.