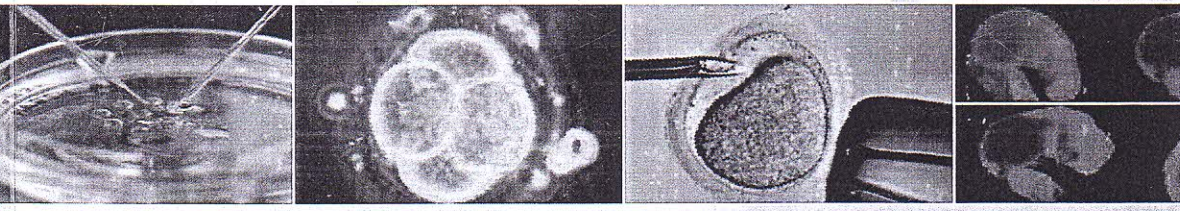
ReProForce
FP7-REGPOT- 2009-1

SEVENTH FRAMEWORK PROGRAMME

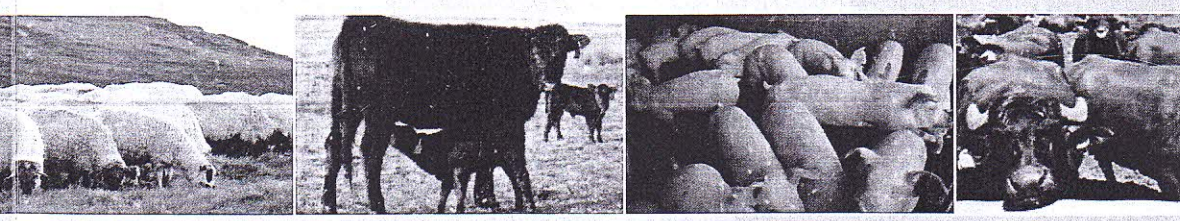
2nd Conference of the Balkan Network for the Biotechnology in Animal reproduction

PROCEEDING

The Conference is dedicated
to 100-th Anniversary
of Acad. K. Bratanov



Institute of Biology and Immunology of Reproduction
"Acad. K. Bratanov", BAS, Sofia, 24 - 25 March, 2011



The International Organizing Committee and Editorial board

Chairman

Professor D. Kacheva	Bulgaria

The Committee members

Associate professor E. Raycheva, PhD	Bulgaria
Associate professor E. Kistanova, PhD	Bulgaria
Professor T. Dovenski	Macedonia
Professor S. Zamfiresku	Romania
Dr. U. Besenfelder , PhD	Austria
Associate professor M. Zujovic, PhD	Serbia
Associate professor G. Michailidis, PhD	Greece
Assistant Professor P. Zrimšek, PhD	Slovenia
Dr. J. Grizelj, PhD	Croatia

The conference is organized in cooperation with the Bulgarian Union of Scientists, section "Animal Husbandry and Veterinary medicine", Sofia.

ISSN 1314-3085

USING OF ULTRASOUND GUIDED OVUM PICK-UP (OPU) IN BOVINE EMBRYO INDUSTRY AS AN ALTERNATIVE TO SUPEROVULATORY TREATMENT

T. Dovenski[1], P. Trojacanec[1], V. Petkov[1], F. Popovska-Percinic[1], Lj. Kocoski[2],
[1]Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje, R. Macedonia;
[2]Faculty of Biotechnical sciences, University St. Kliment Ohridski - Bitola, R. Macedonia;
e-mail: dovenski@fvm.ukim.edu.mk

INTRODUCTION

Bovine Embryo Transfer Industry has undergone a dynamic development in the last 30 years, although first ET in cows was performed only 60 years ago by Willet et al. (1951). Around 100,000 bovine embryos have been flushed from donor cows and transferred every year in Europe and more than 500,000 worldwide. Less than 6% of the total transfers have been carried out using in vitro produced (IVP) embryos, mostly coming from OPU recovered oocytes (Gordon 2003, Merton 2010). Ultrasound guided Ovum pick-up (OPU) is a technique developed for obtaining oocytes for *in vitro* production of embryos by repetitive aspiration of ovarian follicles from live donor cows. Using this technique, usually 2 puncture sessions per week can be performed during several months without hormonal treatment. An average of 5-10 oocytes can be retrieved per session. Costs of hormonal stimulation of superovulation by expensive commercially prepared gonadotropic hormones could be avoided or minimized using OPU, thus the costs per embryo produced would be reduced. OPU also minimizes possible adverse effects on the regular reproductive functions of the treated animals. However, OPU performance requires specialized laboratory equipment and well educated and trained personnel, especially with skills in reproductive ultrasound scanning.

The aim of this paper is to review the current situation in Embryo Production Industry and to address all advantages and possible pitfalls of OPU/IVF methods in comparison to conventional MOET programs.

Multiple ovulation and embryo transfer – from past to present

At the end of the 19th century Walter Heape established scientific fundamentals of embryo transfer by performing the first successful transfer of embryos in rabbits (Betteridge 1981). Half a century later, after ET was also performed in other domestic animals (goats, sheep, pigs), routine ET became possible (viable) in cattle. Initially, the transfer of embryos has been performed for studying reproductive biology. This scientific initiative later on expanded into a possibility for spreading this method for commercial application on the field. Hormonal induction of multiple ovulation and transfer into recipients (usually referred as multiple ovulation and embryo transfer - MOET) has been proposed as a way for accelerating genetic improvement on the breeding herds. For that purpose, at the beginning exogenous gonadotropins such as eCG (Equine chorionic gonadotropin) or hMG (human menopausal gonadotropin) had to be applied. Equine chorionic gonadotropin as a complex glycoprotein with both FSH and LH activity has been shown to have a half-life of 40 h in the cow and persists for up to 10 days in the bovine circulation, causing prolonged follicular growth (Murphy and Martinuk 1991). Due to this „undesired" characteristics eCG was replaced by purified pituitary extracts (porcine, ovine, caprine and recently recombinant bovine FSH). The biological half-life of FSH in the cow has been estimated to be 5 h or less so it must be injected twice a day to successfully

induce superovulation (Monniaux et al. 1983). The „standard" procedure was set as 4 or 5-days treatment, with a total FSH dose of 28 to 50 mg (Armour Units) of a crude pituitary extract (FSH-o, FSH-p) or 400 mg NIH-FSH-PI of the purified pituitary extract, injected twice daily. According the IETS and AETE statistical data, in past decades, on average 5-6 transferable embryos per flushed donor were recovered. However, the main disadvantage of MOET is that outcome of the treatment is very variable: approximately one third of the donors treated did not responded to superovulation, another third produces few embryos and only one third really superovulates giving a large number of embryos (Boland at al. 1991,). Another limitation is the requirement of donors in perfect reproductive conditions which have to be achieved in cows after their lactation peak, since the superovulatory treatment interferes with lactation and induces a decrease in milk production (Galli and Lazzari 2005). Furthermore, the quality and number of transferable ova retrieved from "good responders" vary greatly. It is well accepted that breed, age, nutritional plane and other factors mainly contribute to early regression of corpora lutea or to poor fertilisation due to interrupted sperm transport to the ova (Baldassarre and Karatzas 2004, Basrur and King 2005).

In vitro production (ivp) of embryos

Pioneering attempts for in vitro production of bovine embryos dated from late 70's, parallel to the birth of the first test tube baby Louise Brown in 1978 (Steptoe and Edwards). Brackett et al., 1982, first reported the birth of a calve (named Virgil) after *in vitro* fertilization of an ovulated oocyte. Since then significant revolutionary progress in ET industry has been recorded. Crucial components of the IVP procedure are: in vitro maturation of the oocytes (IVM), in vitro fertilization of maturated oocytes by capacitated sperm (IVF) and in vitro cultivation of embryos (IVC) up to the stage of blastocyst. Oocytes for IVP can be collected by puncturing ovaries obtained from slaughtered animals or by ultrasound-guided transvaginal follicle aspiration in living donor cows (Bols, 2005). Gordon (2003) described in details all aspects of *in vitro* embryo production.

The first MOET experiments in Macedonia have been performed in 1989 (cattle), 1990 (sheep), and 1991 (goats) (Popovski et al. 1990, 1992; Georgievski et al. 1991; Dovenski et al. 1993, 1995; Kocoski et al. 1995). The research activity mainly focused on monitoring of superovulatory response after different hormonal treatments. Our experiments have shown that differences in number of ovulated follicles and quality of obtained embryos depended on the type of hormonal stimulation and the doses, as well as the stage of follicular growth at the beginning of treatment. Own studies revealed that "only" 55% of the large preovulatory follicles finally ovulated, whereas the remaining follicles underwent atresia (Kocoski et al. 1998).

The laboratory for In Vitro Production has been established in 1993, and the first calf produced from an in vitro fertilized frozen-thawed embryo was born in 1997 (Kocoski et al. 1998). In the same year the laboratory was upgraded with equipment for ultrasound guided OPU, which enabled us to produce embryos from oocytes derived from live animals.

Ovum pick up – possibilities and pitfalls

According to Galli and Lazzari (2005), ultrasound guided follicular aspiration, or ovum pick up (OPU) is the most flexible and repeatable technique to produce embryos from live donors (Hasler et al. 1995; Bousquet et al. 1999). This technique was adapted for the use in cattle in 1988 (Pieterse et al.) and subsequently optimized and technically modified (Bols et al., 1995; Bols et al., 1996). Basic equipment consists of a scanner with an adequate vaginal (or adapted for the vaginal use) sector probe equipped including a needle guiding system. The needle is connected to a test tube and to a vacuum pump for aspiration of the follicular fluid which contains the oocytes.

Classical indications for the use of OPU/IVP procedures are genetically high valuable donors with or without non heritable fertility problems such as adhesions, cystic ovaries or low

production rates in MOET programs (De Roover *et al.*, 2005). In addition, (laparoscopic) oocyte retrieval and IVP are possible using pregnant donors (van Wagtendonck-de Leeuw, 2006). OPU can be also performed in prepubertal heifers although the efficiency of this procedure is quite low (Taneja *et al.*, 2000). Optimizing the number of offspring on the female side can even be obtained by combining both MOET and OPU/IVP programmes (Durocher *et al.*, 2006).
If every step in the OPU/IVP procedure is performed under the best conditions, about 80 to 150 embryos can be produced per cow per year (European breeds). Following transfer, this can result in 50 to 70 calves of a single donor cow, which is significantly more than in classic MOET programmes (Hasler *et al.*, 1995; Merton *et al.*, 2003; van Wagtendonck-de Leeuw, 2006). Another advantage of OPU is that it is not necessary to treat the donor with gonadotropins.
However, embryo production from ovum pick up oocytes is very variable and affected by age (Galli et al. 2001), season (Takuma et al. 2010, Viana et al. 2010), FSH stimulation (Chaubal et al. 2006, De Roover et al. 2008, Sendag et al. 2008), donor genetics (Merton et al. 2009). Performing OPU once or twice weekly can result in 1-3 embryos per session (Galli et al. 2001). The possible drawbacks of OPU are higher cost compared to MOET and the requirement of costly aspiration system and specialized laboratory equipment for embryo production.

Opu impact on animal welfare

Ovum pick-up is considered to be a mildly invasive technique employed to retrieve oocytes repeatedly (Pieterse et al. 1988; Bols et al. 1995). Although it does not appear to interfere with subsequent reproductive ability (Broadbent et al. 1997; Kruip and den Daas 1997; Petyim et al. 2000, 2001), it has been demonstrated to affect ovaries and their function. The influence on the ovarian function has been evidenced as irregular cyclic activity (Gibbons et al. 1994; Stubbings and Walton 1995; Boni et al. 1997; Carlin et al. 1999) and slightly modified endocrine patterns (Petyim et al. 2000, 2001). Minor morphological changes of the ovaries as a consequence of OPU have also been recorded, both macroscopically and microscopically (Bergfelt et al. 1994; Gibbons et al. 1994; Kruip et al. 1994; Boni et al. 1997; Petyim et al. 2000, 2001).
Petyim et al. (2006) have reported that dairy heifers subjected to 4 months of twice-weekly OPU did not express any changes in the routine or oestrous behaviour and no signs of clinical disorders have been recorded, except during epidural subprocedure. Similar discussion about possible negative effects of OPU procedure were published by Greve and Jacobsen (2001), summarized as injuries induced to the ovaries and stress experienced during the OPU applications. Besides puncture of the vagina and ovaries, the OPU procedure includes frequent epidural anaesthesia, which may cause pain in the tail, and fused vertebrae (The Farm Animal Welfare Council 1997).

CONCLUSIONS

Ultrasound guided ovum pick-up represents a very flexible biotechnical method that enables repeated recovery of oocytes for IVP which, unlike MOET, does not interfere with the normal reproduction and production cycles of the donor. Furthermore, OPU allows exclusion of the gonadotropine superovulatory treatments, which are often accompanied with inevitable side effects. Its application and combination with IVF procedures in prepubertal animals is appropriate for shortening the generation intervals and hastening genetic improvement.
Even though there is no strongly supported evidence of either a physiological or a behavioural negative response of the donors to the OPU procedure, especially when proper anaesthetic protocols are administered, the public concerns over new biotechnologies that might compromise animal welfare should be our top priority in order to demonstrate the safety of these technologies and the appropriateness for further development and application.

REFERENCES

1. Baldassarre H, Karatzas CN , 2004 -Advanced assisted reproduction technologies (ART) in goats. Anim Reprod. Sci., 82-83, 255-266.
2. Basrur PK, King WA, 2005 -Genetics then and now: breeding the best and biotechnology, Rev sci tech Off int Epiz, 24 (1), 31-49.
3. Bergfelt DR, Lightfoot KC, Adams GP, 1994 -Ovarian synchronization following ultrasound-guided transvaginal follicle ablation in heifers. Theriogenology 42, 895–907.
4. Betteridge K J, 1981 -An historical look at embryo transfer, J Reprod. Fert. 62. 1-13.
5. Boland MP, Goulding D, Roche JF, 1991 -Alternative gonadotrophins for superovulation in cattle, Theriogenology, 35,5-17.
6. Bols PEJ, Langbeen A, Goovaerts IGF, Leroy JLMR, 2010 -Biotechnology of Reproduction in Farm Animals with an Emphasis on Cattle Assisted Reproductive Techniques, Proceed. Int. Cont., Adapting Animal Production to Changes for a Growing Human Population, 45-60.
7. Bols PEJ, Van Soom A, Ysebaert MT, Vandenheede JMM, de Kruif A, 1996 -Effects of aspiration vacuum and needle diameter on cumulus oocyte complex morphology and developmental capacity of bovine oocytes. Theriogenology, 45,1001-14.
8. Bols PEJ, Vandenheede JMM, Van Soom A, de Kruif A, 1995 -Transvaginal ovum pick up (OPU) in the cow: a new disposible needle guidance system. Theriogenology, 43,677-87.
9. Bols PEJ, 2005 -Puncture of immature ovarian follicles in bovine assisted reproduction, Verhandelingen; LXVII:3:177-202.
10. Boni R, Roelofsen MWM, Pieterse MC, Kogut J, Kruip ThAM, 1997 -Follicular dynamics, repeatability and predictability of follicular recruitment in cows undergoing repeated follicular puncture. Theriogenology, 48, 277–289.
11. Bousquet D, Twagiramungu H, Morin N, Brisson C, Carboneau G, Durocher J, 1999 -In vitro embryo production in the cow: an effective alternative to the conventional embryo production approach. Theriogenology, 51,59-70.
12. Brackett BG, Bousquet D, Boice ML, Donawick WJ, Evans JF, Dressel MA, 1982 -Normal development followin in vitro fertilization in the cow. Biol Reprod, 27,147-58.
13. Broadbent PJ, Dolman DF, Watt RG, Smith AK, Franklin MF, 1997 -Effect of frequency of follicular aspiration on the oocyte yield and subsequent superovulatory response in cattle, Theriogenology 47, 1027–1040.
14. Carlin SK, Garst AS, Tarraf CG, Bailey TL, McGilliard ML, Gibbons JR, Ahmadzadeh A, Gwazdauskas FC, 1999 -Effects of ultrasound-guided transvaginal follicular aspiration on oocyte recovery and hormonal profiles before and after GnRH treatment, Theriogenology, 51, 1489–1503.
15. Chaubal SA, Molina JA, Ohlrichs CL, Ferre LB, Faber DC, Bols PE, Riesen JW, Tian X, Yang X, 2006 -Comparison of different transvaginal ovum pick-up protocols to optimise oocyte retrieval and embryo production over a 10-week period in cows, Theriogenology, 65, 1631-1648.
16. De Roover R, Feugang JM, Bols PE, Genicot G, Hanzen C, 2008 -Effects of ovum pick-up frequency and FSH stimulation: a retrospective study on seven years of beef cattle in vitro embryo production. Reprod Dom Anim 43, 239-245.
17. De Roover R, Genicot G, Leonard S, Bols P, Dessy F, 2005 -Ovum pick-up and in vitro embryo production in cows superstimulated with an individually adapted superstimulation protocol. Anim Reprod Sci; 86,13-25.
18. Dovenski T, Kocoski Lj, Popovski K, Veselinovic S, Trojacanec P, Petkov V, Georgievski B, Mickovski G, 1993 -Control of the superovulatory response by ultrasound scanning in cows, 9th Scientific meeting of the A.E.T.E., Abstr. 192.
19. Dovenski T, Trojacanec P, Petkov V, Kocoski Lj, Popovski K, Ralchev I, Lalev I, Sapundjiev E 1995 -Ultrasound observation of the development of follicular population in superovulated ewes, Reproduction & Animal Breeding – Advances and Strategy, Ed. Enne G, Greppi GF, Lauria A, Elsevier, 371-372.
20. Durocher J, Morin N, Blondin P, 2006 -Effect of hormonal stimulation on bovine follicular respons and oocyte developmental competence in a commercial operation. Theriogenology, 65,102-15.
21. Galli C, Crotti G, Notari C, Turini P, Duchi R, Lazzari G, 2001 -Embryo production by ovum pick up from live donors. Theriogenology, 55, 1341-1357.
22. Galli C, Lazzari G, 2005 -Embryo technologies in dairy cattle, The 26th European Holstein and Red Holstein Conference- Proceedings 1-20.
23. Georgievski B, Popovski K, Kocoski Lj, Mrvos G, Dovenski T, Mickovski G, Petkov V, Trojacanec P, 1991 -Embryo Transfer in goats, 1. Summer Conference for ovine and caprine production, 161-166 (in Macedonian).
24. Gibbons JR, Beal WE, Krisher RL, Faber EG, Pearson RE, Gwazdauskas FC, 1994 -Effect of once- versus twice-weekly transvaginal follicular aspiration of bovine oocyte recovery and embryo development. Theriogenology, 42, 405–419.
25. Gordon I, 2003 -Laboratory production of cattle embryos, Second Edition, CABI publishing, Oxon, UK – Cambridge, USA.
26. Greve T, Jacobsen H, 2001 -New embryo-technologies: Implications for animal health and welfare. Arch Tierz, Dummerstorf 44 (Special Issue), 80–90.
27. Hasler JF, Henderson WB, Hurtgen PJ, Jin ZQ, McCauley AD, Mower SA, Neely B, Shuely LS, Stokes JE, Trimmer SA, 1995 -Production, freezing and transfer of bovine IVF embryos and subsequent calving results. Theriogenology; 43,141-152.
28. Japan Livestock Technology Association, 1995. Manual of bovine embryo transfer, 1-44.
29. Kocoski Lj, Dovenski T, Trojacanec P, Petkov V, Popovski K, Mickovski G, Mickov Lj, 1998 -The first calves produced from IVF procedure in Macedonia. Macedonian Journal of Reproduction, 4 (2), 93-98.
30. Kocoski Lj, Popovski K, Dovenski T, Petkov V, Trojacanec P, Georgievski B, Mickovski G, 1995 -In vitro production of bovine embryos, Macedonian Journal of Reproduction 1,2, 29-34.
31. Kruip ThAM, den Daas JHG, 1997 -In vitro produced and cloned embryos: Effects on pregnancy, parturition and offspring. Theriogenology, 47, 43–53.
32. Merton JS, Ask B, Onkundi DC, Mullaart E, Colenbrander B, Nielen M, 2009 -Genetic parameters for oocyte number and embryo production within a bovine ovum pick-up-in vitro production embryo-production program. Theriogenology, 72, 885-893.
33. Merton JS, de Roos APW, Mullaart E, de Ruigh L, Daal L, Vos PLAM, Dieleman SJ, 2003 -Factors affecting oocyte quality and quantity in commercial application of embryo technologies in cattle breeding industry. Theriogenology 59,651-74.
34. Merton S, 2010 -National Statistical Data of Bovine Embryo Transfer Activity in Europe, Proceedings of 26th AETE scientific meeting, 33-76.
35. Monniaux D, Chupin D, Saumande J, 1983 -Superovulatory responses of cattle, Theriogenology, 19,55-82.
36. Murphy BD, Martinuk SD, 1991 -Equine chorionic gonadotropin, Endocrine Reviews, 12, 27-44.
37. Petyim S, Bage R, Forsberg M, Rodriguez-Martinez H, Larsson B, 2000 -The effect of repeated follicular puncture on ovarian function in dairy heifers. J Vet Med A 47, 627–640.
38. Petyim S, Bage R, Forsberg M, Rodriguez-Martinez H, Larsson B, 2001 -Effects of repeated follicular puncture on ovarian morphology and endocrine parameters in dairy heifers. J Vet Med A, 48, 449–463.
39. Pieterse MC, Kappen KA, Kruip ThAM, Taverne MAM, 1988 -Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. Theriogenology- 30:751-62.
40. Popovski K, Kocoski Lj, Trojacanec P, Dovenski T, Petkov V, Veselinovic S, Georgievski B, Mickovski G, Adamov M, 1992 -Ultrasonic examination of the ovarian responce in donor ewes. 8th Scientific meeting of the A.E.T.E., Abstr., 200.
41. Popovski K, Veselinovic S, Kocoski Lj, Mickovski G, Georgievski B, Dovenski T, Danev M, Petkov V, 1990 -Comparive results of superovulatory treatment of cows-embryo donor, stimulated by FSH and PMSG - with or withour neutra/PMSG, Macedonian veterinary review, 1-2, 7-14 (in Macedonian).
42. Sendag S, Cetin Y, Alan M, Hadeler KG, Niemann H, 2008: -Effects of eCG and FSH on ovarian response, recovery rate and number and quality of oocytes obtained by ovum pick-up in Holstein cows. Anim Reprod Sci 106, 208-214.
43. Steptoe PC, Edwards RG. 1978 -Birth after reimplantation of a human embryo. The Lancet, 2:366.
44. Stubbings RB, Walton JS, 1995 -Effect of the ultrasonicallyguided follicle aspiration on oestrous cycle and follicular dynamics in Holstein cows. Theriogenology, 43,705–712.
45. Takuma T, Sakai S, Ezoe D, Ichimaru H, Jinnouchi T, Kaedei Y, Nagai T, Otoi T, 2010 -Effects of season and reproductive phase on the quality, quantity and developmental competence of oocytes aspirated from Japanese black cows. J Reprod Dev, 56, 55-59.
46. Taneja M, Bols PEJ, Van de Velde A, Ju J-C, Schreiber D, Tripp MW, Levine H, Echelard Y, Riesen J, Yang X., 2000 -Developmental competence of juvenile calve oocytes in vitro and in vivo: influence of donor animal variation and repeated gonadotropin stimulation. Biol Reprod, 62,206-13.
47. van Wagtendonk-de Leeuw AM, 2006 -Ovum pick up and in vitro production in the bovine after use in several generations: a 2005 status. Theriogenology, 65, 914-25.
48. Viana JH, Palhao MP, Siqueira LG, Fonseca JF, Camargo LS, 2010,-Ovarian follicular dynamics, follicle deviation, and oocyte yield in Gyr breed (Bos indicus) cows undergoing repeated ovum pick-up. Theriogenology, 73, 966-972.
49. Willett EL, Black WG, Casida LE, Stone WH, Buckner PJ, 1951 -Successful transplantation of a fertilized bovine ovum. Science, 113, 247.