collection of embryos at a younger age by means of ovum pickup (OPU) is beneficial for our breeding program. Healthy 9- to 10-month-old Holstein-Friesian animals were selected at young age based on their maturity, (i.e. OPU could be performed by normal standard procedure/equipment). Animals were not stimulated with hormones. Oocytes were collected by OPU once every week during a period of ~8 weeks. Collected oocytes were matured, fertilized, and cultured for 7 days in SOF culture medium according to standard procedures (Merton *et al.* 2002). Embryo development was scored at Day 7. Results were analysed by Student's *t*-test. On average 11.7 oocytes were collected from animals as young as 9 months of age. However, embryo development of the oocytes from young animals was only 0.4 embryo per session at Day 7 (3% embryo development). When only the results of the first OPU session were taken into account, 18.2 oocytes and 0.8 embryo could be collected per animal per session. Whether an animal was observed in oestrus before the first OPU session affected results. Animals that had shown clear signs of oestrus before the first OPU session produced significantly more embryos than animals that did not (Table 1). It is concluded that OPU is possible on animals at 9 to 10 months of age but only when animals have been in oestrus before the first OPU session. Best results were obtained for the first OPU session.

Table 1.   Effect of first oestrus before OPU on embryo production

Oestrus	No. of animals	No. of embryos (total)	Mean no. of embryos per animal
No	24	5	0.2[a]
Yes	11	23	2.1[b]

[a,b]Values in columns with different superscripts are significantly different, $P < 0.05$.

241   EFFECT OF PRODUCTION EFFICIENCY IN THE LIKELIHOOD OF PREGNANCY OF *IN VITRO*-DERIVED BOVINE EMBRYOS

L. F. Feres[A], L. S. A. Camargo[B], M. P. Palhao[C], F. Z. Brandao[A], and J. H. M. Viana[B,C]

[A]Universidade Federal Fluminense, Niteroi, RJ, Brazil;
[B]Embrapa, Juiz de Fora, MG, Brazil;
[C]Universidade de Alfenas, Alfenas, MG, Brazil

Improving *in vitro* culture systems to optimize embryo yield has been a major research goal. The relationship between the efficiency of embryo production systems and the pregnancy outcomes, however, remain controversial. The aim of the present study was to evaluate the likelihood of pregnancy of *in vitro*-produced embryos derived from batches with different relative efficiency indexes. Data of 702 ovum pick-up (OPU) and *in vitro* embryo production (IVEP) sessions, and of 2456 embryo transfers, recorded from 2008 to 2012, were evaluated. All donors were from the same herd, and were of the same breed (Gir, *Bos indicus*), as well as the semen used for IVF. The cumulus-oocyte complex (COC) recovery and IVEP were performed by the same team, in a single IVF laboratory, and using standard medium and procedures. Only data from embryos transferred as fresh were used, and records from 97 OPU/IVEP sessions in which no embryo was produced, or embryos were frozen or discharged due to lack of recipients, were discharged. The remaining 605 sessions were stratified in quartiles (I to IV, each one corresponding to 25% of total data) according to COC production of the donors, or stratified in ranges (0–25%, 26–50%, 51–75%, and 76–100%) according to COC quality (percentage of viable COC or of grade I COC) and to embryo production efficiency endpoints (cleavage rate, blastocyst rate). Pregnancy rates were compared among quartiles or ranges by the chi-square method. On average, the Gir donors produced 24.8 ± 0.6 COC per OPU, from which 14.4 ± 0.4 were classified as viable (57.8%), and 3.2 ± 0.1 as grade I (12.9%). On average 6.1 ± 0.2 embryos (morulas and blastocysts) were produced per OPU per donor, and mean pregnancy rate was 30.9%. As expected, donors with greater total COC yield (quartile I) also produced more viable oocytes (25.5 ± 0.7 v. 15.7 ± 0.3, 10.5 ± 0.2 and 5.8 ± 0.2), more COC grade I (4.8 ± 0.4 v. 3.9 ± 0.3, 2.6 ± 0.2 and 1.6 ± 0.1), and more embryos (9.0 ± 0.4 v. 6.9 ± 0.3, 5.0 ± 0.2 and 3.3 ± 0.1) than donors from quartiles II, III, or IV, respectively ($P < 0.0001$). Nevertheless, there was no difference ($P > 0.05$) in pregnancy rates for embryos produced from donors ranked in the different quartiles (30.9 v. 29.3, 31.5, and 30.5% for quartiles I to IV, respectively). Similarly, there was no difference ($P > 0.05$) in the pregnancy rate of embryos derived from OPU sessions in which there was a high or low percentage of viable or grade I COC. *In vitro* production efficiency (cleavage and blastocyst rates) also had no effect ($P > 0.05$) on further pregnancy rates. In conclusion, these results suggest that there is no relationship among the average number or quality of the COC recovered by OPU, the efficiency of IVEP, and the likelihood of pregnancy of *in vitro*-derived embryos.

242   PROMISING TWO-STEP EVALUATION SYSTEM FOR SELECTING HIGH DEVELOPMENTAL COMPETENCE *IN VITRO* FERTILIZED EMBRYOS IN CATTLE USING WELL-OF-THE-WELL DISH

M. Taniai, M. Takayama, O. Dochi, and K. Imai

Department of Dairy Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

Bovine IVF embryos are evaluated morphologically using light microscopy just before transfer. However, this evaluation method is subjective, and an objective method with more certainty is needed. Sugimura *et al.* (PLoS ONE 2012 7, e36627) reported a promising system for selecting healthy IVF bovine embryo by using time-lapse cinematography and 5 prognostic factors. This study was to investigate the efficacy of a 2-step evaluation system of IVF embryos using microscopy for selecting high developmental competence IVF embryos. Cumulus-oocyte complexes (COC) were

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