VETERINARSKI ARHIV 80 (4), 445-454, 2010

The developmental competence of bovine immature oocytes and quality of embryos derived from slaughterhouse ovaries or live donors by ovum pick up

Martina Karadjole[1]*, Iva Getz[1], Marko Samardžija[1], Nino Maćešić[1], Mario Matković[2], Zdenko Makek[1], Tugomir Karadjole[1], Goran Bačić[1], Tomislav Dobranić[1], and Marko Poletto[3]

[1]*Clinic for Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

[2]*Center for Reproduction and Animals Breeding of Croatia, Zagreb, Croatia*

[3]*Postgraduate specialist student, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

KARADJOLE, M., I. GETZ, M. SAMARDŽIJA, N. MAĆEŠIĆ, M. MATKOVIĆ, Z. MAKEK, T. KARADJOLE, G. BAČIĆ, T. DOBRANIĆ, M. POLETTO: The developmental competence of bovine immature oocytes and quality of embryos derived from slaughterhouse ovaries or live donors by ovum pick up. Vet. arhiv 80, 445-454, 2010.

ABSTRACT

The aim of this research was to compare the developmental competence of oocytes recovered from slaughterhouse ovaries (SO) and those recovered *in vivo* by ovum pick up (OPU). OPU was performed in 6 donor heifers synchronized with $PGF_2\alpha$ and stimulated with pFSH, twice a day for two days. OPU was repeated once a week for six consecutive weeks. The control group consisted of slaughterhouse-derived oocytes. The number of aspirated follicles, retrieved oocytes and oocyte recovery rate were recorded. Oocytes of grade 1 and 2 were matured, fertilized and cultured *in vitro* in SOFaaBSA until day 9. The cleavage rates on day 2, the total number of morulas and blastocysts on day 7 and the numbers of hatched blastocysts on day 9 were recorded. Differential staining of the inner cell mass (ICM) and trophectoderm cells (TE) were performed on a random sample of day 7 blastocysts (n = 24). Significantly more follicles were aspirated from SO (19.3 versus 11.5, $P<0.05$). This was reflected in a significantly higher number of oocytes collected from the same group (12.6 versus 6.5, $P<0.05$). A higher proportion of OPU-derived oocytes reached the morula/blastocyst stage at day 7 (44.7% vs. 29.9%, $P<0.05$) and hatched blastocyst stage at day 9 (35.6% vs. 16.8%, $P<0.05$). OPU-derived embryos displayed significantly higher number ($P<0.05$) of ICM cells which was also reflected to higher proportion of ICM cells. The results demonstrate that oocytes recovered *in vivo* after OPU are more competent to develop to the blastocyst stage than those derived from slaughterhouse ovaries.

Key words: heifer, oocyte, developmental competence

*Corresponding author:

Martina Karadjole, PhD, DVM, Clinic for Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia, Phone: +385 1 2390 326; Fax: +385 1 2441 390; E-mail: mlojkic@vef.hr

ISSN 0372-5480
Printed in Croatia

Introduction

Oocyte quality is often defined as the competence to yield a blastocyst within an *in vitro* production system (MERTON et al., 2003). Numerous pieces of research suggest that the intrinsic quality of the oocyte is the critical factor determining the proportion of oocytes developing to the blastocyst stage (LONERGAN et al., 2003). The developmental competence of oocytes is acquired gradually and increases with follicular development. It is related to the physiological and reproductive status of the donor animals, follicle size and the extent and integrity of cumulus cell investment (LONERGAN et al., 1994; LIEBFRIED-RUTLEDGE, 1996), culture conditions, reference semen used and the oocyte collection method (slaughterhouse versus *in vivo* collection).

The oocytes for *in vitro* production of bovine embryos could be harvested from slaughterhouse ovaries as well as from live donor cows. Slaughterhouse derived ovaries contain a highly heterogeneous population of oocytes that are recovered regardless of follicular dynamics and may come from cattle with diverse reproductive backgrounds. Production of embryos from slaughterhouse ovaries begins with oocytes collected from all follicles visible on the surface of the bovine ovary. Oocytes obtained from slaughtered animals are variable in their developmental competence after *in vitro* maturation (IVM) (BILODEAU-GOESEELS and PANICH, 2002). One of the main disadvantages of this technology is the lack of repeatability. Once the cow has been slaughtered there can be no further collection of oocytes from the same donor. Oocytes are more frequently recovered from live animals by transvaginal ultrasound-guided oocyte collection or Ovum pick-up. In conjunction with *in vitro* embryo production (IVP), this technique provides an alternative means to increase the number of offspring from genetically valuable cows (GALLI et al., 2001). Oocytes collected by OPU originate from follicles that lack dominance and have not undergone final preovulatory development. As with slaughterhouse derived oocytes, final maturation has to take place *in vitro*.

Numerous differences have been reported between slaughterhouse and OPU-derived oocytes. The distribution of quality classes may differ between slaughterhouse and OPU-derived oocytes with more good quality oocytes collected from slaughterhouse ovaries (MULLAART et al., 1999). Also, the outcome of IVP studies can be related to those differences (MERTON et al., 2003).

The aim of this research was to compare the developmental competence of immature oocytes recovered from slaughterhouse ovaries (SO) and those recovered *in vivo* by ovum pick up (OPU) in *in vitro* production system.

Materials and methods

Oocyte aspiration from slaughterhouse ovaries. Bovine ovaries (n = 72) were obtained from a local slaughterhouse and were transported to the laboratory in physiological saline

with antibiotics (100 I.U. penicillin and 100 µg streptomycin/mL) at 37 °C within 3 h of slaughter. There was no available information regarding the health or physiological status of donors except that they were Simmental or Holstein-Friesian heifers. Cumulus-oocyte complexes (COCs) were aspirated from 2 to 8 mm diameter follicles using an 18G needle attached to a vacuum pump. The aspirated follicular fluid was transferred from the collection tube to a Perti dish (Intergrid®, Falcon). The oocytes were counted and classified into four grades according to the appearance of the surrounding cumulus cells and ooplasm (MAKEK et al., 1998): grade 1: oocytes completely surrounded with more than three layers of cumulus cells and evenly granulated ooplasm that completely filled the zona pellucida; grade 2: oocytes with one to three cell layers and with evenly granulated ooplasm; grade 3: partially denuded oocytes with unevenly granulated ooplasm; grade 4: completely denuded oocytes, degenerated oocytes, expanded oocytes. Only Grade 1 and 2 oocytes were submitted to IVM while grades 3 and 4 were discarded.

*Oocyte aspiration from live cows.*Transvaginal ultrasound guided oocyte collection was performed in 6 healthy, cycling Simmental donor heifers aged between 16-24 months. The donors were synchronized with two prostaglandin $F_2\alpha$ treatments at 11-day intervals. The gonadotropine treatment was initiated between 9 and 10 days after induced oestrus. Treatment consisted of pFSH (Folltropin®, Bioniche Animal Health, Ireland), twice a day, for two days. The total amount of pFSH administered was 200 mg NIH-FSH-P1 in 4 equivalent doses. Ultrasound-guided Ovum Pick-Up was performed 48 hours after the last FSH injection. Superovulation and OPU were repeated once a week for six consecutive weeks.

The OPU procedure and oocyte recovery. Oocyte collection using OPU technology was performed according to the standard procedure described by PIETERSE et al. (1988) and GETZ (2004). Briefly, xylazine hydrocloride was administered i.m. (Xyilapan, Chassot; 0.25 mL/100 kg) to all animals, following an epidural anaesthesia (4 mL of 2% lidocaine hydrocloride) prior to transvaginal aspiration of oocytes. A 7.5 MHz sector transvaginal transducer of an ultrasound scanner (Pie Medical, the Netherlands) mounted on a holder with a grip was used to guide the needle (18G disposable needle, attached to the stainless steel guidance) through the vagina into the ovary. All visible follicles (>2mm) were punctured and the contents collected in a sterile 15 mL tube (Falcon™) with aspiration medium (TCM hepes supplemented with antibiotics, heparine and bovine serum albumine) attached to a suction pump to ensure constant aspiration pressure. The follicular fluid obtained by aspiration was transferred into an embryo transfer filter (Minitub™, Germany). The recovered oocytes were identified under a stereomicroscope, counted and morfologically classified into four categories as mentioned earlier. The number of aspirated follicles, the number of retrieved oocytes and oocyte recovery rate were recorded.

In vitro embryo production. Oocytes recovered from slaughterhouse ovaries or live donors were cultured separately. Recovered oocytes were transferred into a culture dish with TCM hepes supplemented with 10% of FCS. Oocytes of grade 1 and 2 with intact cumulus investment and evenly granulated cytoplasm were matured *in vitro* in TCM 199 bicarbonate medium supplemented with 10% foetal calf serum, FSH/LH (Pergonal, 75/75 IU, Serono), 1 μg/mL estradiol-17ß and 100 μM cysteamine. Oocytes were incubated in groups of 10 in 50 μL droplets of maturation media under mineral oil for 24 h at 38.5 °C with 5% CO_2 in humidified air. The expanded COCs were washed in HEPES-TALP medium supplemented with 6 mg/mL bovine serum albumine (BSA) and transferred into 40 μL droplets of fertilization medium under mineral oil. *In vitro* fertilization (IVF) was performed in modified Tyrode's bicarbonate buffered solution supplemented with 10 μg/mL heparin, 5 μg/mL hypotaurine, 5 μg/mL epinephrine and 6 mg/mL BSA. In all experiments, frozen semen from the same bull was used. Sperm preparation for IVF on BoviPure® gradient was accomplished according to SAMARDŽIJA et al. (2006). The final concentration was adjusted to 1×10^6 spz/mL. Incubations were carried out at 39 °C in 5% CO_2 in air for 24 h. After the sperm-oocytes co-incubation, the presumptive zygotes were denuded from the cumulus cells and spermatozoa and washed in HEPES-TALP medium and in culture medium. Fertilized oocytes were cultured *in vitro* (IVC) in synthetic oviductal fluid (SOF) with amino acids and 8 mg/mL BSA without glucose for 48 h and then transferred to SOF with 1.5 mM glucose. The embryos were cultured *in vitro* until day 9 at 39 °C in 5% CO_2, 5% O_2 and 90% N_2 atmosphere with maximum humidity. Cleavage was assessed 48 hours post insemination (hpi) and blastocyst development was recorded on days 7 and 9 (day 0 = day of IVF). Evaluation of embryos was performed under a stereomicroscope using morphological criteria according to the International Embryo Transfer Society (IETS) (WRIGHT, 1998).

Differential staining of blastocysts. Differential staining of the inner cell mass (ICM) and trophectoderm cells was performed on random samples on day 7 blastocysts (n = 24).

The zona pellucida of intact blastocysts was removed by treatment with 0.5% pronase. Zona-free embryos were washed five times in phosphate buffered saline containing 0.1% polyvynil alcohol. Embryos were then incubated in a 30:70 dilution of rabbit anti-bovine whole serum in TCM 199 bicarbonate at 39 °C for 1 h. After washing in PBS 0.1% PVA, the embryos were incubated in a 1:4 dilution of a guinea pig complement in TCM 199 bicarbonate supplemented with 10 μg/mL propidium iodide (PI) for 1 h. The embryos were then briefly washed in ice-cold TCM 199 Hepes supplemented with 10 μg/mL PI and fixed in ice-cold absolute ethanol. After fixation, the embryos were transferred for 3-5 minutes to 10 μg/mL bisbenzimide (Hoechst 33343) in absolute alcohol at room temperature. Presumptive stained blastocysts were transferred to a drop

of glycerol on a microscopic slide and covered with a cover slip. Embryos were examined under a fluorescence microscope (Olympus, Tokyo, Japan) equipped with an UV filter. Bisbenzimide-stained inner cell mass nuclei labelled with bisbenzimide appeared blue and trophectoderm nuclei labelled with both bisbenzimide and PI appeared red or pink. The ICM and TE nuclei were counted under the microscope.

Statistical analysis. The statistical analyses of the treatments were performed using STATA 6.0 (STATA Corp., USA). Data were analyzed by Chi-square test or Mann-Whitney and Kruskall-Wallis test.

Results

A total of 469 oocytes in 6 independent replicates were aspirated from 689 follicles of 36 slaughtered heifers. For the six donor heifers, a total of 419 follicles were punctured during six OPU sessions and 250 oocytes were collected.

Significantly more follicles were aspirated from slaughterhouse ovaries (19.3 versus 11.5, $P<0.05$). This was reflected in the significantly higher number of oocytes collected from the same group (12.6 versus 6.5, $P<0.05$) (Table 1). The results of slaughtered heifers were presented per animal (n = 36) and from live donors per OPU session (n = 36).

Table 1. Number of follicles punctured and oocytes recovered from slaughterhouse ovaries or donor heifers by ovum pick up

Oocyte origin	n	No. of follicles punctured	No. of oocytes recovered	Oocyte recovery rate (%)	No. of grade 1-2 oocytes
Slaughtered heifers	36	19.3 ± 1.1^{a}	12.6 ± 0.92^{a}	68.5 ± 0.09^{a}	10.8 ± 0.76^{a}
Donor cows	6	11.5 ± 0.82^{b}	6.5 ± 0.62^{b}	59.5 ± 0.06^{b}	5.7 ± 0.43^{b}

Values with different superscripts within the same columns differ significantly

From the total of 496 oocytes collected from slaughterhouse ovaries, only grade 1 and 2 oocytes (n = 398) were matured, fertilized and cultured *in vitro* for 9 days. In donor heifers, from a total of 250 oocytes, grade 1 and 2 oocytes (n = 212) were submitted to IVM/IVF/IVC (Table 2). No significant difference was observed in terms of cleavage rate between slaughterhouse and OPU-derived oocytes. However, a significantly higher proportion ($P<0.05$) of OPU-derived oocytes reached the morula/blastocyst stage at day 7 (44.7% versus 29.9%) and hatched blastocyst stage at day 9 (35.6% versus 16.8%).

A random sample of grade 1 blastocysts (n = 12) from each group was selected for differential staining (Table 3). No significant difference was observed in total cell number between slaughterhouse and OPU-derived oocytes. However, OPU-derived oocytes displayed a significantly higher number ($P<0.05$) of ICM cells which was also reflected in

the higher proportion of ICM cells in OPU-derived oocytes, compared to slaughterhouse-derived oocytes.

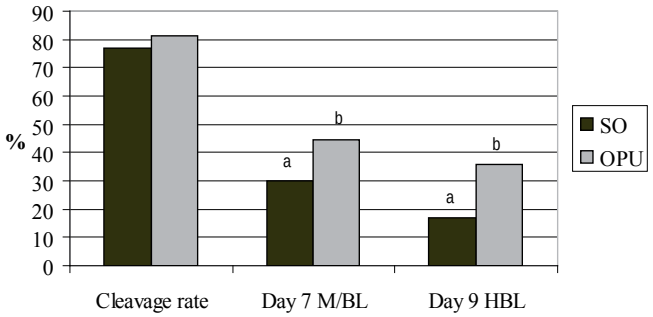

Fig. 1. Effect of bovine oocyte origin on *in vitro* development of embryos. Values with different superscripts differ significantly.

Table 2. Effect of bovine oocyte origin on embryo quality (mean ± SD)

Oocyte origin	Day 7 Blastocyst n	Total cells (n ± SD)	ICM	
			(n ± SD)	Proportion (%)
Slaughterhouse oocytes	13	123.77 ± 11.44[a]	32.22 ± 3.12[a]	26.05 ± 0.8[a]
OPU-derived oocytes	11	141.00 ± 11.53[a]	41.56 ± 4.11[b]	29.36 ± 1.16[b]

Discussion

The work presented here shows that a higher mean number of follicles were punctured and oocytes recovered from the slaughterhouse ovaries than from the donor heifers. CAROLAN et al. (1994) reported a mean of 13.5 oocytes recovered per slaughterhouse ovary, which conforms with our results of 12.6 oocytes recovered from slaughterhouse ovaries. By transvaginal aspiration we collected 6.5 oocytes per cow which is similar to reports of LOONEY et al. (1994) and GETZ (2004). In the OPU-IVP practice, the source of oocytes is determined by the resolution of the equipment used and OPU session interval (MERTON et al., 2003). This means that only follicles larger than 2 mm can be punctured. In contrast, follicles of all sizes (≥1 mm) visible on the surface of the slaughterhouse ovary can be punctured, which contributes to the higher number of follicles punctured and oocytes recovered from slaughterhouse ovaries.

The success of OPU is partly measured by the recovery rate (RR), which is influenced by hormonal pre-treatment of the animals (PIETERSE et al., 1992; GETZ, 2004), puncture frequency (GIBBONS et al., 1994), vacuum level used, the needle (BOLS et al., 1995) and operator experience (SCOTT et al., 1994). As a result, recovery rates vary between 7% (SCOTT et al., 1994) and 69.6% (LOONEY et al., 1994), for different research teams. We attained a recovery rate of 59.5% in OPU group. The recovery rate of slaughterhouse-derived oocytes was significantly higher (68.5%).

Differences between slaughterhouse and OPU-derived oocytes have previously been reported with controversial results (GETZ et al., 2001; MERTON et al., 2003; LOPES et al., 2006; KARADJOLE et al., 2007; MERTON et al., 2008). LOPES et al. (2006) reported a higher blastocyst yield from slaughterhouse-derived oocytes than those aspirated by OPU. Such differences are probably related to the quality of OPU-retrieved oocytes, which is often lower than the quality of slaughterhouse-derived oocytes. HASHIMOTO et al. (1999) have reported that extrinsic factors such as vacuum pressure and needle diameter used during aspiration influence the morphological characteristics of the recovered oocytes. MULLART et al. (1999) reported that cumulus oocyte complexes become less tightly connected to the follicle because of post mortem effect and as a consequence slaughterhouse-derived oocytes are collected with more complete cumulus investment. Also, morphological selection of OPU-derived oocytes prior to IVM is very rare in contrast to the pre-selection to slaughterhouse derived oocytes where only grade 1 and 2 oocytes are submitted for further procedure. This may contribute to the better blastocyst yields obtained from slaughterhouse-derived oocytes.

In our study the blastocyst rate from OPU-derived oocytes was higher than those aspirated from slaughterhouse ovaries. We used the same morphological selection for both slaughterhouse and OPU-derived oocytes where only grade 1 and 2 oocytes retrieved from slaughterhouse ovaries or by OPU were submitted to IVM, while poor quality oocytes were discarded. Furthermore, the time between collecting the oocytes and placing them into the maturation medium was longer for slaughterhouse than for OPU-derived oocytes, which could negatively affect the developmental competence of oocytes. These two factors could influence the better blastocyst yield obtained with OPU-derived oocytes. Follicle size could also have an impact on the blastocyst rate because the majority of oocytes collected from slaughterhouse ovaries in our study originated from small follicles, which have impaired developmental competence compared to large follicles (BLONDIN and SIRARD, 1995; KARADJOLE, 2009).

Our study also demonstrated that OPU-derived blastocysts displayed a significantly higher number of ICM cells ($P<0.05$) compared to slaughterhouse-derived oocytes. The ratio of the number of inner and outer cells in blastocysts is an important factor for normal development. A minimal number of ICM-cells are required to obtain pregnancy while

excessive allocation of cells to the TE might be responsible for pregnancy anomalies, recorded after transferring *in vitro* produced embryos (HASLER, 1998). VAN SOOM et al. (1997) demonstrated that blastocysts produced at day 6 after fertilization had a significantly higher number of ICM cells than blastocysts, which developed at day 7 or 8. This may be the reason for the higher ICM count in OPU-derived blastocysts since our previous study (KARADJOLE, 2009) demonstrated that OPU-derived embryos develop faster to the blastocyst stage than embryos originated from slaughterhouse ovaries.

In conclusion, these results demonstrate that the oocytes recovered *in vivo* after OPU are more competent to develop to the blastocyst stage than those derived from slaughterhouse ovaries.

References

BILODEAU-GOESEELS, S., P. PANICH (2002): Effects of oocyte quality on development and transcriptional activity in early bovine embryos. Anim. Reprod. Sci. 71, 143-155.

BLONDIN, P., M. A. SIRARD (1995): Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. Mol. Reprod. Dev. 41, 54-62.

BOLS, P. E. J., A. VAN SOOM, A. DE KRUIF (1995): Transvaginal ovum pick up (OPU) in the cow: a new disposable needle guidance system. Theriogenology 43, 677-687.

CAROLAN, C., P. MONAGHAN, M. GALLAGHER, I. GORDON (1994): Effect of recovery method on yield of bovine oocytes per ovary and their developmental competence after maturation, fertilization and culture *in vitro*. Theriogenology 41, 1061-1068.

GALLI, C., G. CROTTI, C. NOTARI, P. TURINI, R. DUCHI, G. LAZZARI (2001): Embryo production by ovum pick up from live donors. Theriogenology 55, 1341-1357.

GETZ, I., M. MATKOVIĆ, Z. MAKEK, M. LOJKIĆ, A. TOMAŠKOVIĆ, T. KARADJOLE (2001): *In vitro* developmental competence of embryos derived from transvaginal ultrasound oocyte aspiration in cycling cows and from slaughterhouse ovaries. ESDAR Newsletter 6: Proceedings of the 5th Annual Conference of the European Society for Domestic Animal Reproduction. 13-15. September. Vienna, Austria. p. 27.

GETZ, I. (2004): The efficacy of ovarian stimalation in donor cows on the resuts of ovum pick-up and *in vitro* embryo production. Dissertation. Faculty of Veterinary Medicine. Zagreb, Croatia.

GIBBONS, J. R., W. E. BEAL, R. L. KRISHER, E. G. FABER, R. E. PEARSON, F. C. GWAZDAUSKAS (1994): Effects of once versus twice weekly transvaginal follicular aspiration on bovine oocyte recovery and embryo development. Theriogenology 42, 405-419.

HASHIMOTO, S., R. TAKAKURA, M. KISHI, T. SUDO, N. MINAMI, M. YAMADA (1999): Ultrasound-guided follicle aspiration: the collection of bovine cumulus-oocyte complexes from ovaries of slaughtered or live cows. Theriogenology 51, 757-765.

HASLER, J. F. (1998): The current status of oocyte recovery, *in vitro* embryo production and embryo transfer in domestic animals, with an emphasis on the bovine. J. Anim. Sci. 76 Suppl. 3, 52-74.

KARADJOLE, M. (2009): The effect of follicle size on oocyte quality and embryo development after *in vitro* fertilization. Dissertation. Faculty of Veterinary Medicine. Zagreb, Croatia.

KARADJOLE, M., I. GETZ, M. SAMARDŽIJA, M. MATKOVIĆ, Z. MAKEK, T. KARADJOLE, G. BAČIĆ, N. MAČEŠIĆ, T. DOBRANIĆ (2007): The effect of origin of the immature oocytes on *in vitro* developmental competence of bovine embryos. Proceedings of the 23th Anual Meeting of European Embryo Transfer Association, 7-8 September. Alghero, Italy. pp. 180.

LIEBFRIED-RUTLEDGE, M. L. (1996): Factors determining competence of *in vitro* produced cattle embryos. Theriogenology 51, 473-485.

LONERGAN, P., D. RIZOS, T. FAIR, M. P. BOLAND (2003): *In vitro* production of bovine embryos: factors affecting blastocyst yield and quality. Proceedings of the 4th Middle-European Buiatric Congress, 23-27 April. Lovran, Croatia. pp. 33-38.

LONERGAN, P., P. MONAGHAN, D. RIZOS, M. P. BOLAND, I. GORDON (1994): Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization and culture *in vitro*. Mol. Reprod. Dev. 37, 48-53.

LOONEY, C. R., B. R. LINDSEY, C. L. GONSETH, D. L. JONSON (1994): Commercial aspects of oocyte retrieval and *in vitro* fertilization (IVF) for embryo production in problem cows. Theriogenology 41, 67-72.

LOPES, A. S., T. MARTINUSSEN, T. GREVE, H. CALLESEN (2006): Effect of days post-partum, breed and Ovum pick-up scheme on bovine oocyte recovery and embryo development. Reprod. Dom. Anim. 41, 196-203.

MAKEK, Z., I. GETZ, M. CERGOLJ, M. HERAK, A. TOMAŠKOVIĆ, T. DOBRANIĆ, V. SUŠIĆ (1998): Selection of immature bovine oocytes as the preliminary phase of *in vitro* fertilization. Vet. Arhiv 68, 109-119.

MERTON, J. S., A. P. DE ROOS, E. MULLAART, L. DE RUIGH, L. KAAL, P. L. A. M. VOS (2003): Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. Theriogenology 59, 651-674.

MERTON, J. S., T. OTTER, B. AERTS, E. MULLAART (2008): Effect of oocyte collection method and breed on efficiency of oocyte collection and subsequent *in vitro* bovine embryo production. Proceedings of the 24th Anual Meeting European Embryo Transfer Association, 12-13 September. Pau, France. pp. 208.

MULLAART, E., A. VERBRUGGE, B. AERTS, J. S. MERTON (1999): Optimization of OPU procedure. Proceedings of the 15th Scientific Meeting of European Embryo Transfer Association, 10-11 September. Lyon, France. pp. 216.

PIETERSE, M. C., K. A. KAPPEN, Th. A. M KRUIP, M. A. M. TAVERNE (1988): Aspiration of bovine oocytes during transvaginal ultrasound scaning of ovaries. Theriogenology 30, 751-762.

PIETERSE, M. C., P. L. A. M. VOS, Th. A. M. KRUIP, Y. A. WURTH, Th.T. VAN BENDEN, A. H. WILLEMSE, M. A. M. TAVERNE (1992): Repeated transvaginal ultrasound-guided ovum pick-up in ECG treated cows. Theriogenology 37, 273.

SAMARDŽIJA, M., M. KARADJOLE, M. MATKOVIĆ, M. CERGOLJ, I. GETZ, T. DOBRANIĆ, A. TOMAŠKOVIĆ, J. PETRIĆ, J. ŠURINA, J. GRIZELJ, T. KARADJOLE (2006): A comparison of BoviPure® and Percoll® on bull sperm separation protocols for IVF. Anim. Reprod. Sci. 91, 237-247.

SCOTT, C. A., L. ROBERTSON, R. T. D. DE MOURA, C. PATERSON, J. S. BOYD (1994): Technical aspects of transvaginal ultrasound-guided follicular aspiration in cows. Vet. Rec. 134, 440-443.

VAN SOOM, A., M. T. YSEBAERT, A. DE KRUIF (1997): Relationship between timing of development, morula morphology and cell allocation to inner cell mas and trophectoderm in *in vitro* produced bovine embryos. Mol. Reprod. Dev. 47, 47-56.

WRIGHT, J. M. (1998): Photographic illustrations of embryo developmental stage and quality codes. In: Manual of the International Embryo Transfer Society, 3rd ed. (Stringfellow, D. A., S. M. Siedel, Eds.). IETS, Savoy, Illinois. pp. 167-170.

Received: 15 July 2009
Accepted: 22 December 2009

KARADJOLE, M., I. GETZ, M. SAMARDŽIJA, N. MAĆEŠIĆ, M. MATKOVIĆ, Z. MAKEK, T. KARADJOLE, G. BAČIĆ, T. DOBRANIĆ, M. POLETTO: Razvojna sposobnost nezrelih goveđih jajnih stanica i kvaliteta zametaka podrijetlom od klaoničkih jajnika i živih davateljica. Vet. arhiv 80, 445-454, 2010.

SAŽETAK

Svrha ovog istraživanja bila je usporediti razvojnu sposobnost jajnih stanica dobivenih iz klaoničkih jajnika i junica davateljica postupkom transvaginalne aspiracije folikula. Jajne stanice uzete su od šest junica davateljica sinkroniziranih s $PGF_2\alpha$ i stimuliranih FSH-om, dva puta dnevno tijekom dva dana. Postupak je ponavljan jednom tjedno tijekom šest uzastopnih tjedana. Paralelno su punktirani folikuli jajnika junica uzeti na liniji klanja. Bilježen je broj aspiriranih folikula, broj aspiriranih jajnih stanica i uspjeh aspiracije. Jajne stanice morfološki su ocijenjene te je praćena njihova sposobnost dozrijevanja, oplodnje i uzgoja *in vitro*. Bilježen je postotak brazdanih jajnih stanica 2. dana, broj morula/blastocista 7. dana i broj izlegnutih blastocista 9. dana uzgoja *in vitro*. Diferencijalno bojenje stanica zametnoga čvorića i trofoblasta izvedeno je na nasumce odabranim blastocistama 7. dana uzgoja *in vitro*. Iz klaoničkih jajnika aspirirano je značajno više folikula (19,3 prema 11,5, $P<0,05$) i jajnih stanica (12,6 prema 6,5, $P<0,05$) u usporedbi s davateljicama. Kod davateljica uzgojeno je više morula i blastocista 7. dana (44,7% prema 29,9%, $P<0,05$) i izlegnutih blastocista 9. dana uzgoja *in vitro* (35,6% prema 16,8%, $P<0,05$). Diferencijalnim bojenjem ustanovljen je značajno veći broj stanica zametnoga čvorića ($P<0,05$) u blastocistama podrijetlom od davateljica. Rezultati istraživanja pokazuju da jajne stanice dobivene postupkom transvaginalne ultrazvučne aspiracije imaju veću sposobnost razvoja do stadija blastociste u uvjetima uzgoja *in vitro* od klaoničkih jajnih stanica.

Ključne riječi: junica, jajna stanica, razvojna sposobnost