

Comparison of different synchronization treatments in donor heifers for embryo production *in vitro*

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GETZ, I., M. KARADJOLE, M. MATKOVIĆ, T. KARADJOLE, G. BAČIĆ, Z. MAKEK, J. GRIZELJ, M. CERGOLJ, N. MAČEŠIĆ: Comparison of different synchronization treatments in donor heifers for embryo production *in vitro*. Vet. arhiv 81, 545-556, 2011.

ABSTRACT

The aim of the study was to compare the effect of different synchronization protocols on oocyte retrieval and blastocyst production *in vitro*. Twelve Simmental heifers were randomly allocated to one of three synchronization groups. In the 1st group heifers were synchronized with two PGF₂α injections in 11-days interval. In the 2nd group heifers were synchronized the same way and dominant follicles (DF) were removed 36 hours before pFSH stimulation. In the 3rd group a progestin ear implant was inserted for 10 days and DF were aspirated 36 hours before pFSH stimulation. The same stimulation protocol with pFSH, starting on Day 9/10 of cycle, was used in all treatment groups. Ovum pick-up (OPU) was performed 48 hours after the last pFSH injection. The number of aspirated follicles, number and quality of retrieved oocytes were recorded. Oocytes were matured, fertilized and cultured *in vitro* in SOFaaBSA until Day 9. The mean DF diameter and the mean number of expanded oocytes showed a tendency towards significance ($0.05 \leq P \leq 0.10$) in the progestin treatment group. No significant differences between treatment groups were observed in terms of cleavage rate on Day 2, morula/blastocyst rate on Day 7 and hatched blastocysts rate on Day 9. The results indicated that progestin treatment did not prevent the establishment of follicular dominance on the ovaries of heifers. Furthermore, the synchronization protocol did not affect the number of follicles and the recovered oocytes, or the number of *in vitro* produced embryos.

Key words: heifer, synchronization, oocyte, *in vitro* culture

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Introduction

The *in vitro* production (IVP) of bovine embryos involves the collection of oocytes by OPU which are further processed by *in vitro* maturation, fertilization and culture (PIETERSE et al., 1991). These techniques offer an alternative for producing offspring from genetically superior dams, pregnant cows and heifers, post-partum cows and even from prepuberal calves (GETZ et al., 2000; GALLI et al., 2001). The success of OPU and *in vitro* embryo production depends considerably on the number and the quality of retrieved oocytes per session and the culture conditions. The average yield of OPU is rather limited: 5 to 8 oocytes per cow per session (HENDRIKSEN et al., 2004) and varies significantly and repetitively between donor cows, ranging from 0 to 26 oocytes per collection (WAGTENDONK-De LEEUW, 2006). On average, 20 to 30% of OPU oocytes develop into transferable embryos at Day 7 (WAGTENDONK-De LEEUW, 2006). Different extrinsic (BOLS, 1997) and intrinsic oocyte factors (LONERGAN et al., 2003; RIZOS et al., 2005) can interfere with oocyte recovery rate, fertilization and development to the blastocyst stage *in vitro*. The most significant improvement with respect to improving oocyte yield and quality and subsequent embryo production was hormonal pre-stimulation prior to OPU using gonadotropins (FRY et al., 1994). Oocyte competence was increased when the period between pFSH administration and OPU was extended by 48 h, so-called “coasting” of the follicular wave, to provide a more favourable follicular environment for the oocytes to complete their final maturation (BLONDIN et al., 2002). The population of oocytes used in OPU-IVF is more homogenous compared to multiple ovulation and embryo transfer (MOET), due to repetitive sessions resulting in the elimination of dominant and atretic follicles. Consequently, the length of the period between the two sessions significantly affects the embryo production rate (MERTON et al., 2003). A significantly higher number of cumulus oocytes complexes (COCs) were collected over a 7-day compared to a 3- or 4-day interval. However, the quality of the COCs on the basis of the cumulus investment is highest with a 3-day interval and lowest with a 7-day interval (MERTON et al., 2003). The presence of the dominant follicle emerging approximately 3 days after OPU, through its inhibin and estradiol secretion, has an inhibitory effect on the development of other follicles (MAPLETOFT et al., 2002). In order to increase the quality and homogeneity of the oocyte population at the start of superstimulation treatment, several groups have studied the effect of dominant follicle removal (DRF) and follicular wave synchronization on *in vitro* embryo production. BLONDIN et al. (2002) demonstrated that removal of DF and a 48 hour coasting period following LH administration is sufficient to produce the maximum number of competent oocytes in FSH stimulated heifers. HENDRIKSEN et al. (2004) proved the negative effect of the dominant follicle presence on the developmental competence of oocytes after prostaglandin or progestin oestrus synchronization. RAMOS et al. (2010) indicated that progestin treatment only had a modest effect in suppressing

FSH concentration and new follicular wave emergence, and failed to prevent or delay the establishment of follicular dominance when OPU was performed at 7 day intervals.

The aim of the present study was to compare the effect of different synchronization protocols and dominant follicle removal on oocyte retrieval and blastocyst *in vitro* production in pFSH stimulated Simmental heifers.

Materials and methods

Experimental design and synchronization protocols. Twelve cyclic Simmental heifers, aged between 14 and 20 months (body weight: 380 - 470 kg) were selected from a feedlot, based on transrectal palpation of their ovaries. During the study they were kept in tie stalls, fed hay, concentrate and mineral supplement and given water *ad libitum*. Throughout the experimental period (6 months) all animals were in good condition. After 14 days of adaptation, heifers were randomly allocated into three treatment groups (four animals per group). The experimental design was a three-treatment crossover study, in that every heifer received each of the three synchronization treatments in sequence (in total: n = 12 per treatment). All animals had a 4-week rest period (or at least one oestrus cycle) between subsequent treatments. The treatment groups, treatment protocols and experimental designs are summarized in Fig. 1 and Fig. 2. Oestrus synchronization protocols were started independently of cycle stage in all treatment groups.

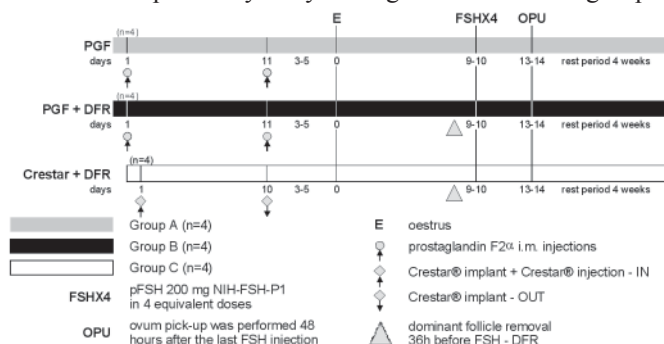


Fig. 1. The treatment groups and oestrus synchronization protocols in FSH stimulated Simmental heifers for OPU/IVF

In the PGF group heifers were synchronized with two prostaglandin F_{2α} analogue injections (Estrumate[®], 2 mL i.m. Shering.Plough Ltd.) at 11-days interval. Heifers underwent gynaecological examination (vaginal and rectal examination) 48 and 72 h after the last PGF_{2α} injection, to confirm the oestrus and ovulation after 96 h. On day 8 of the synchronized oestrus cycle (where day 0 was the day of oestrus) the presence

of dominant follicle (diameter ≥ 8 mm) and corpus luteum was registered by transrectal ultrasonography (Scanner LC 100 Vet, Pie Medical, Netherlands with the 6/8 MHz linear array transrectal probe).



Fig. 2. Synchronization protocol of OPU/IVF oocytes donors - crossover design

In the PGF + DFR group the heifers were synchronized as described for previous group and dominant follicles, if registered by transrectal ultrasonography, were aspirated on day 8 of the synchronized oestrus cycle i.e. 36 hours before pFSH stimulation.

In the Crestar + DFR group a 3 mg norgestomet subcutaneous implants (Crestar[®], Intervet-International B.V.) were inserted under the convex surface of the ear for 10 days. On the day of implant insertion, heifers received an i.m. injection of 3 mg norgestomet and 3.8 mg estradiol valerate (Crestar[®] injection, Intervet-International B.V.). As in the previous treatment groups dominant follicles were aspirated on Day 8 of the synchronized oestrus cycle.

Ovarian stimulation and oocyte recovery. The stimulation with pFSH (Folltropin[®]-V, Vetrepharm Inc., London, Canada) was the same for all three synchronization groups and subsequent repetitions (OPU 1, OPU 2 and OPU 3). Superovulatory treatment was initiated between Days 9 and 10 after induced oestrus and consisted of pFSH twice a day, for two days. The total amount of pFSH administered was 200 mg NIH-FSH-P1 in 4 equivalent doses. OPU was performed 48 hours after the last FSH injection.

Transvaginal ultrasound guided oocyte collection was performed using an ultrasound scanner with a sector 5/7.5 MHz transducer (Pie Medical, Netherlands), attached to an aspiration pump and ovum pick-up needle (Terumo 18G) guidance system, as described by BOLS (1997) and GETZ (2004). Prior to follicular aspiration, xylazine hydrochloride was administered i.m. (Xylapan, Chassot; 0.25 mL/100 kg) to all animals, following epidural anaesthesia (4 mL of 2% lidocaine hydrochloride). During OPU sessions, all follicles >3 mm were counted and aspirated into a 10 mL sterile tube, containing aspiration media consisting of TCM 199, buffered with Hepes and supplemented with heparine and Bovine Serume Albumine (BSA). Aspiration pressure was 80 mm Hg, at a flow rate equivalent to 12 mL/min. Retrieved COCs were classified in five quality categories:

- (1) Oocytes completely surrounded by compact follicular cell layers (more than 3) and with an evenly granulated ooplasm that completely filled the *zona pellucida* (G1);

- (2) Oocytes partially surrounded with the compact cumulus cell layer with less than 3 cell layers and with an evenly granulated ooplasm (G2) ;
- (3) Oocytes incompletely surrounded with less compact cumulus cells and with unevenly granulated ooplasm (G3) ;
- (4) “Nude” oocytes with degenerated ooplasm with vacuoles, fragmented, or with the remains of the ooplasm that did not completely fill the *zona pellucida* (G4).
- (5) Expanded oocytes with unevenly granulated or degenerated ooplasm (G5).

In vitro embryo production. Grade 1, 2 and 3 oocytes were matured *in vitro* in TCM 199 bicarbonate medium supplemented with 10% fetal calf serum, FSH/LH (Pergonal® 75/75 I.U., Serono), 1 µg/mL estradiol-17β and 100 µM cysteamine for 24 hours 39°C and with 5% CO₂ in air. Matured oocytes were fertilized *in vitro* with frozen/thawed bull semen prepared on BoviPure® gradient according to SAMARDŽIJA et al. (2006a) and SAMARDŽIJA et al. (2006b). The final concentration was adjusted to 1×10⁶ spz/mL. Incubations were carried out at 39 °C in 5% CO₂ in air for 24 h. Presumptive zygotes were cultured in Synthetic Oviduct Fluid with amino acids and 8 mg/mL BSA and without glucose (SOFaaBSA). After 48 hours they were transferred to SOFaaBSA with 1.5 mM glucose and cultured *in vitro* until Day 9 (Day 0 = IVF). The medium was changed every second day. Incubation was performed at 39 °C in the atmosphere of 5% CO₂, 7% O₂ and 88% N₂. Cultured embryos were evaluated morphologically according to IETS standards (ANONYM., 1998).

Statistical analysis. Data obtained during the experiments were analyzed by the mixed model procedure (StatSoft, Statistic, version 7.1.). The model included the comparison of data from a single animal over time and then a comparison of data between treatment groups. Continuous variables were analyzed by ANOVA. The results of repeated measurements over time were compared by the Duncan multi-stage test to evaluate the normality and the homogeneity of variances. A significant difference between treatment groups was declared at P<0.05, and a tendency towards significance was assumed when 0.05≤P≤0.10.

Results

The overall results are derived from 36 OPU sessions performed on 12 donor Simmental heifers: 3 sessions per each animal following three different synchronization treatments. A total of 292 oocytes in 3 subsequent OPU sessions were aspirated from 440 follicles in 12 Simmental heifers. No significant differences were observed in terms of follicle number and oocyte recovery results between subsequent OPU sessions.

The effect of different synchronization protocols on oocyte retrieval results in pFSH stimulated Simmental heifers are shown in Table 1. There was no difference between the

treatment groups in terms of the proportion of the follicles and oocytes number in each category ($P > 0.10$).

Table 1. Effect of different synchronization protocols on oocyte retrieval in pFHS stimulated Simmental heifers (mean \pm SEM)

Synchronization protocol	No. of follicles punctured	No. of oocytes recovered	Oocyte recovery rate (%)	No. of oocytes in IVP
PGF group (n = 12)	11.6 \pm 0.93	8.0 \pm 0.92	68.2 \pm 0.05	6.7 \pm 0.92
PGF + DFR group (n = 12)	12.9 \pm 1.23	8.0 \pm 1.17	59.7 \pm 0.05	6.8 \pm 1.23
Crestar + DFR group (n = 12)	12.2 \pm 0.66	8.3 \pm 0.74	67.6 \pm 0.03	6.1 \pm 0.75

In the PGF group a dominant follicle, 10.05 \pm 0.07 mm in diameter, was registered in 8 out of 12 heifers 36 hours before ovarian pFHS stimulation. In the PGF + DRF group a dominant follicle, 10.16 \pm 0.07 mm in diameter, was registered and aspirated in 8 heifers, 36 hours before ovarian pFHS stimulation, and in the Crestar + DRF group in 7 heifers a DF 12.71 \pm 0.11 mm in diameter was registered. The mean diameter of the dominant follicle in this group showed a tendency toward significance ($0.05 \leq P \leq 0.10$) with regard to the PGF group of heifers. No significant differences in OPU outcome, in terms of follicles and retrieved oocytes numbers, were observed within groups and over groups between animals with or without a DF present on ovaries 36 h prior to pFHS treatment.

The mean number of collected cumulus-oocyte complexes and the results of their categorization after three treatment protocols are presented in Table 2.

Table 2. The mean number (\pm SEM) of COCs collected after pFHS ovarian stimulation and the mean number (\pm SEM) of different oocytes categories after three treatment protocols

Synchronization protocol	No. of oocytes recovered	G1 + G2	G3	G4	Exp.oocytes
PGFx2 group (n = 12)	8.0 \pm 0.92	5.3 \pm 0.67	1.4 \pm 0.39	0.3 \pm 0.18	1.0 \pm 0.21
PGF + DFR group (n = 12)	8.0 \pm 1.17	5.6 \pm 1.03	1.2 \pm 0.32	0.6 \pm 0.22	0.6 \pm 0.36
Crestar + DFR group (n = 12)	8.3 \pm 0.74	5.1 \pm 0.75	1.0 \pm 0.3	0.8 \pm 0.29	1.4 \pm 0.43*

* $0.05 \leq P \leq 0.10$

The mean number of expanded oocytes in the Crestar + DRF group showed a tendency toward significance ($0.05 \leq P \leq 0.10$) with regard to the PGF and PGF + DRF groups of heifers.

A total of 235 oocytes collected from donor heifers in all three treatment groups were matured, fertilized and cultured *in vitro* for 9 days (Fig. 3). No significant differences between treatment groups were observed in terms of cleavage rate on day 2, morula/blastocyst rate on day 7 and hatched blastocysts rate on day 9.

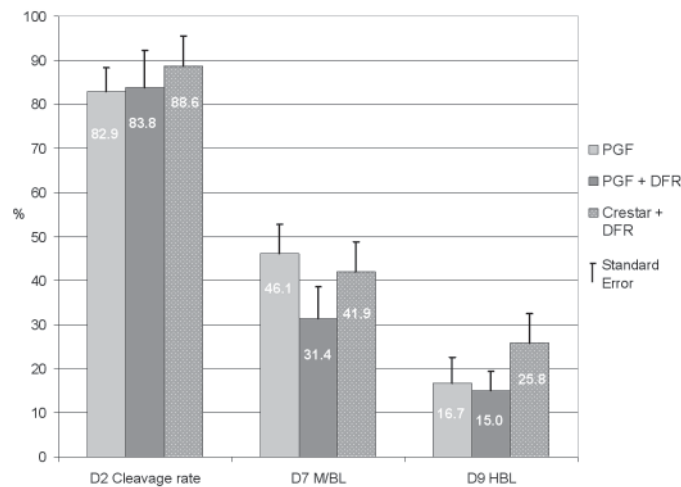


Fig. 3. Effect of synchronization treatment in OPU/IVF donor heifers on *in vitro* development of embryos

Discussion

In the present crossover study three different synchronization treatments, of which two included dominant follicle removal 36 h prior to pFSH ovarian stimulation, were compared in twelve Simmental heifers, to assess the number, quality and IVP developmental competence of the retrieved oocytes. There was no significant difference in the number of follicles aspirated and oocytes recovered between subsequent OPU sessions within the treatment groups and between the groups. In addition, no differences between the synchronization treatment groups were established: in the PGF group recovery rate (RR) was 68.2.5%, in the PGF + DRF group 59.7% and in the Crestar + DRF group 67.6 %.

However, other studies have revealed that oocyte recovery rate, quality and developmental competence in OPU/IVF are influenced by different technical and biological factors. Technical factors that have been investigated included vacuum pressure, needle diameter (BOLS, 1997), scanner resolution and needle guidance system (MULLAART et al., 1999) and operator experience (SCOTT et al., 1994). Biological factors investigated include the origin of the oocytes (KARADJOLE et al., 2007) and donor animal herself (FERRET

et al., 2006; MERTON et al., 2008), hormonal pre-stimulation (GETZ, 2004; CHAUBAL et al., 2007), timing and frequency of OPU sessions (BLONDIN et al., 2002; PETYIM et al., 2003) follicular wave synchronization and dominant follicle removal (GARCIA et al., 2000; CHAUBAL et al., 2006).

The follicular wave in the current study was initiated through the synchronization treatment in all three groups and by dominant follicle removal 36 hours before pFSH stimulation in two treatment groups. In the Crestar + DRF group, the dominant follicles had a larger diameter and a higher mean number of expanded oocytes (not suitable for IVP) was recorded, showing a tendency towards significance with regard to the PGF and PGF + DRF groups of heifers. Our results are in agreement with data published by other authors in which different ways for initiation of the follicular wave were used, in order to increase the quality and homogeneity of COSs at the start of a superstimulation treatment. The effect of the DF on COC quality was reported by SALAMONE et al. (1999). COCs at Day 5 and 6 of the follicular wave had a higher incidence of expanded cumulus than COCs at Day 1-2- or 3-4 of the follicular wave. In this study, follicular waves were initiated by DFR in heifers at random stages of the oestrus cycle and COCs were collected after slaughter. However, in spite of the higher incidence of COCs with expanded cumulus, the oocytes at Day 5-6 of the cycle gave a higher production of morulae (26%) than the oocytes of earlier stages (14%).

The results of a preliminary study (GETZ et al., 2007) demonstrated that the synchronization protocol did not affect the number of follicles following superstimulation, but it did affect the number and the quality of the retrieved oocytes and the blastocysts yield. Significantly more oocytes for IVP were retrieved from FSH stimulated Simmental heifers synchronized with two PGF injections (9.5 ± 5.6) than in the norgestomet group (5.9 ± 3.6). The observation made in that study supported the findings of RAMOS et al. (2010) that norgestomet treatment did not prevent the establishment of follicular dominance and did not improve the number and quality of the recovered oocytes. After the norgestomet synchronization protocol they recovered 5.8 ± 0.5 oocytes per heifer per OPU session without FSH stimulation.

In the present study every heifer received each of the three synchronization treatments (PGF, PGF + DRF, Crestar + DRF) in subsequent repetitions (OPU 1, OPU 2 and OPU 3) following FSH stimulation of ovaries. No significant differences between the treatment groups were observed in terms of cleavage rate on Day 2, morula/blastocyst rate on Day 7 and hatched blastocysts rate on Day 9.

REIS et al. (2002) collected COCs from FSH stimulated Simmental heifers at 15 (OPU1) and 21 (OPU2) days following PGF synchronization, on four consecutive occasions at 15-week intervals. More COCs were collected during OPU1 than OPU 2 (7.2 ± 0.47 versus 5.7 ± 0.44) but the respective percentages of good quality (categories 1

and 2) did not differ significantly ($55 \pm 3\%$ versus $47 \pm 3\%$) and also blastocyst yield from good quality COCs ($24 \pm 3\%$ and $26 \pm 4\%$). De ROOVER et al. (2005a) used a three day pFSH stimulation protocol 48 h after DFR and norgestomet implant insertion. The mean number of punctured follicles per session was 11.9 ± 7.7 , with 16% of follicles exceeding 11 mm. These follicles yielded a mean of 5.6 ± 4.1 COCs, 32% of which had ≥ 3 layers of cumulus cells (quality 1 and 2), resulting in a mean of 2.0 ± 2.3 blastocysts on Day 7.

The conflicting results of our preliminary and the present studies could be influenced by the individual response of donor heifers to FSH stimulation. In the retrospective study of OPU/IVF results obtained following 665 sessions conducted in 112 animals, De ROOVER et al. (2005b) demonstrated the variability of response to gonadotrophin stimulation. In “low, high and medium responder” categories different results were obtained for: mean follicle number (8.8 ± 4.8 , 22.4 ± 10.5 , 13.2 ± 5.2), COC numbers (6.3 ± 3.9 , 18.5 ± 8.2 , 10.4 ± 4.0) and also recovery rate (72%, 83% and 79%) and percentage embryo development (29%, 30% and 24%).

In conclusion, these results indicated that progestin treatment did not prevent the establishment of follicular dominance on the ovaries of heifers. Furthermore, the synchronization protocol did not affect the number of follicles and the number and quality of the recovered oocytes, or the number of *in vitro* produced embryos.

References

- ANONYMOUS (1998): Manual of the International Embryo Transfer Society, 3rd ed. Savoy, IL: IETS.
- BLONDIN, P., D. BOUSQUET, H. TWAGIRAMUNGU, F. BARNES, M. A. SIRARD (2002): Manipulation of follicular development to produce developmentally competent bovine oocytes. Biol. Reprod. 66, 38-43.
- BOLS, P. E. J. (1997): Transvaginal Ovum Pick-Up in the Cow: Technical and Biological Modifications. Doctoral Thesis, University of Ghent, Belgium.
- CHAUBAL, S. A., J. A. MOLINA, C. L. OHLRICH, L. B. FERRE, D. C. FABER, P. E. J. BOLS, J. W. RIESEN, X. TIAN, X. YANG (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.
- CHAUBAL, S. A., L. B. FERRE, J. A. MOLINA, D. C. FABER, P. E. J. BOLS, P. REZAMAND, X. TIAN, X. YANG (2007): Hormonal treatments for increasing the oocyte and embryo production in an OPU-IVP system. Theriogenology 67, 719-728.
- De ROOVER, R., G. GENICOT, S. LEONARD, P. BOLS, F. DESSY (2005a): Ovum pick-up and *in vitro* embryo production in cows superstimulated with an individually adapted superstimulation protocol. Anim. Reprod. Sci. 86, 13-25.

- De ROOVER, R., P. E. J. BOLS, G. GENICOT, C. HANZEN (2005b): Characterisation of low, medium and high responders following FSH stimulation prior to ultrasound-guided transvaginal oocyte retrieval in cows. *Theriogenology* 63, 1902-1913.
- FERRET, S., B. GRIMARD, A. A. PONTER, C. JOLY, C. PONSARD, P. HUMBLLOT (2006): Reduction of body-weight gain enhances *in vitro* embryo production in overfed superovulated dairy heifers. *Reproduction* 131, 783-794.
- FRY, R. C., T. L. SIMPSON, T. J. SQUIRES, R. A. PARR, R. M. DAMANIK (1994): Factors affecting transvaginal oocyte pick-up in heifers. *Theriogenology* 41, 197 (abstr.).
- 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.
- GARCIA, J. M., R. Z. PUELKER, K. B. AVELINO, R. VANTINI, C. F. M. RODRIGUEZ, C. R. ESPER (2000): Effect of follicular wave synchronization method on an ovum pick-up program. *Theriogenology* 53, 354 (abstr.).
- GETZ, I. (2004): The efficiency of ovarian stimulation in cows used for transvaginal ultrasound aspiration of oocytes and culture *in vitro* of bovine embryos. Dissertation. Faculty of Veterinary Medicine. Zagreb, Croatia.
- GETZ, I., M. MATKOVIĆ, M. LOJKIĆ, Z. MAKEK, G. BAČIĆ, M. CERGOLJ (2000): Transvaginal ovum pick-up in the cow: alternative in the production of bovine embryos for transfer. *Vet. arhiv* 70 (Suppl.). S75-S80.
- GETZ, I., M. KARADJOLE, M. SAMARDŽIJA, Z. MAKEK, M. MATKOVIĆ, T. DOBRANIĆ, M. CERGOLJ (2007): Comparison of different synchronization protocols on OPU/IVF results in FSH stimulated simmental heifers. Proceedings of the 23th Annual Meeting of European Embryo Transfer Association, 7-8 September. Alghero, Italy, 170.
- HENDRIKSEN, P. J. M., W. N. M. STEENWEG, J. C. HARKENA, J. S. MERTON, M. M. BEVERS, P. L. A. M. VOS, S. J. DIELEMAN (2004): Effect of different stages of the follicular wave on *in vitro* developmental competence of bovine oocytes. *Theriogenology* 61, 909-920.
- 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 Annual Meeting of European Embryo Transfer Association, 7-8 September. Alghero, Italy, 180.
- 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 Buiatrics Congress, 23-27 April. Lovran, Croatia, 33-38.
- MAPLETOFT, R. J., K. BENNETT STEWARD, G. P. ADAMS (2002): Recent advances in the superovulation in cattle. *Reprod. Nutr. Dev.* 42, 601-611.
- 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

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- production. Proceedings of the 24th Annual Meeting European Embryo Transfer Association, 12-13 September. Pau, France, 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. p.p. 216.
- PETYIM, S., R. BÄGE, T. HALLAP, A.-S. BERGQUIST, H. RODRIGUEZ-MARTINEZ, B. LARSSON (2003): Two different schemes of twice-weekly ovum pick-up in dairy heifers: effect on oocyte recovery and ovarian function. *Theriogenology* 60, 175-188.
- PIETERSE, M. C., P. L. A. M. VOS, Th. A. M KRUIP, Y. A. WURTH, Th. H. van BENEDEN, A. H. WILLEMSE (1991): Transvaginal ultrasound guided follicular aspiration of bovine oocytes. *Theriogenology* 35, 19-24.
- RAMOS, A. F., R. RUMPF, J. U. CAMARA, M. R. MOLLO, I. PIVATO, A. P. MARQUES, R. SARTORI (2010): Effect of follicular wave synchronization on *in vitro* embryo production in heifers. *Anim. Reprod. Sci.* 117, 201-207.
- REIS, A., M.E. STAINES, R.G. WATT, D.F. DOLMAN, T.G. McEVORY (2002): Embryo production using defined oocyte maturation and zygote culture media following repeated ovum pick-up (OPU) from FSH-stimulated Simmental heifers. *Anim. Reprod. Sci.* 72, 137-151.
- RIZOS, D., L. BURKE, P. DUFFY, M. WADE, J. F. MEE, K. J. O'FARELL, M. MacSIURTAİN, M. P. BOLAND, P. LONERGAN (2005): Comparison between nulliparous heifers and cows as oocyte donors for embryo production *in vitro*. *Theriogenology* 63, 939-949.
- SALAMONE, D. F., G. P. ADAMS, R. J. MAPLETOFT (1999): Changes in the cumulus-oocyte complex of subordinate follicles relative to follicular wave status in cattle. *Theriogenology* 52, 549-561.
- SAMARDŽIJA, M., M. KARADJOLE, I. GETZ, Z. MAKEK, M. CERGOLJ, T. DOBRANIĆ (2006b): Effects of bovine spermatozoa preparation on embryonic development *in vitro*. *Reprod. Biol. Endocrinol.* 4, 58.
- SAMARDŽIJA, M., M. KARADJOLE, M. MATKOVIĆ, M. CERGOLJ, I. GETZ, T. DOBRANIĆ, A. TOMAŠKOVIĆ, J. PETRIĆ, J. ŠURINA, J. GRIZELJ, T. KARADJOLE (2006a): 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.
- WAGTENDONK-De LEEUW, A. M. (2006): Ovum pick up and *in vitro* production in the bovine after use in several generations. A 2005 status. *Theriogenology* 65, 914-925.

Received: 13 July 2010

Accepted: 29 October 2010

GETZ, I., M. KARADJOLE, M. MATKOVIĆ, T. KARADJOLE, G. BAČIĆ, Z. MAKEK, J. GRIZELJ, M. CERGOLJ, N. MAČEŠIĆ: Usporedba postupaka sinkronizacije estrusa junica davateljica jajnih stanica u proizvodnji zametaka *in vitro*. Vet. arhiv 81, 545-556, 2011.

SAŽETAK

Svrha ovog istraživanja bila je usporediti utjecaj različitih metoda sinkronizacije estrusa na razvojnu sposobnost jajnih stanica i uzgoj *in vitro* govedih zametaka. Za davateljice jajnih stanica odabrane su junice u ciklusu simentalne pasmine (n=12), koje su potom nasumce podijeljene u tri skupine. Prva skupina junica sinkronizirana je pomoću dvije injekcije PGF₂α u razmaku od 11 dana. U drugoj je skupini nakon sinkronizacije s PGF₂α aspiriran dominantni folikul (DF) 36 sati prije stimulacije jajnika s pFSH. Treća skupina junica sinkronizirana je pomoću gestagenskih ušnih usadaka tijekom 10 dana, a DF je aspiriran 36 sati prije pFSH stimulacije jajnika. U svih je davateljica aktivnost jajnika stimulirana pomoću pFSH, počevši od 9/10 dana ciklusa. Transvaginalna aspiracija jajnih stanica izvedena je 48 sati nakon zadnje injekcije pFSH. Zabilježen je broj aspiriranih folikula, broj aspiriranih jajnih stanica i uspjeh aspiracije. Jajne stanice dobre kvalitete uzete su u postupak dozrijevanja, oplodnje i uzgoja *in vitro*. Prosječni promjer DF i broj ekspaniranih jajnih stanica loše kvalitete pokazivao je tendenciju prema značajnosti ($0,05 \leq P \leq 0,10$) u skupini junica sinkroniziranih gestagenima. Postotak brazdanih jajnih stanica drugoga dana uzgoja, morula i blastocista sedmoga dana uzgoja te postotak izlegnutih blastocista devetoga dana nije se statistički razlikovao između pojedinih skupina. Rezultati istraživanja pokazuju da sinkronizacija junica pomoću gestagenskih usadaka nije spriječila nastanak dominantnog folikula na jajnicima davateljica. Postupak sinkronizacije ciklusa nije utjecao na broj folikula i aspiriranih jajnih stanica niti na rezultate uzgoja govedih zametaka *in vitro*.

Ključne riječi: junica, sinkronizacija, jajna stanica, uzgoj *in vitro*
