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Ovum pick-up in cattle: a 25 yr retrospective analysis

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Abstract

Repeated oocyte collection by transvaginal ultrasound-guided follicular puncture (Ovum Pick-Up: OPU), implicitly associated to *in vitro* embryo production (IVEP), has become alternative and competitive to superovulation for embryo production in cattle. It is alternative because it can be applied successfully irrespective of the reproductive status of the donor, *i.e.* in pregnant and acyclic animals, in those having patent tube or genital tract infections and in animals insensitive to superovulatory treatment. It is competitive because it can yield more transferable embryos per donor on a monthly basis. Through the years, the number of transferable embryos provided by OPU has significantly increased mainly due to the technological improvement of IVEP. However, limits to OPU application remain due to lower pregnancy rate of *in vitro vs. in vivo* produced embryos or non optimal cooperation between OPU practitioners and IVF laboratories. This review will focus on the technical modifications proposed for improving OPU efficiency, the analysis of the physiological parameters that affect OPU and IVEP efficiency and, finally, the use of OPU as a tool to study and manipulate reproductive activity in cattle.

Keywords: bovine, follicle dynamics, *in vitro* embryo production, Ovum Pick-Up.

Introduction

One of the main limitations of *in vitro* embryo production technologies (IVEP) as developed in livestock was the impossibility to repeat the collection of competent oocytes from the same individual. In fact, 1) the use of ovaries from slaughtered animals as oocyte source permits the recovery of a very limited number of gametes with respect to the potential oocyte population contained in the ovary; 2) only oocytes contained within follicles involved in the follicular wave dynamics have the proper developmental competence that is required for IVEP. The collection of oocytes from living individuals might overcome this limitation.

In human, where *in vivo* oocyte collection represents the only reasonable possibility to get the female gamete, this methodology has moved very quickly. In light of this evidence, most of the new methodologies proposed in live animals represent adaptations to novelties which have been developed in human. Of course, these techniques have been adapted in relation to their use and application in animals.

Repeated *in vivo* oocyte collection in cattle was first performed by Canadian researchers who used endoscopy via the right paralumbar fossa (Lambert *et al*., 1983). A total of 129 laparoscopies were performed in 50 heifers. Eight animals underwent more than four interventions. Occasional adhesions were observed, but they never interfered with ovary examination and follicular aspiration. The rate of oocyte recovery was higher when a suction device was used rather than a syringe system. The use of a 19-gauge (G) needle with a 45-degree bevel and a vacuum pressure of 250 mmHg provided the best results.

An ultrasonically guided aspiration of bovine follicular oocytes was first proposed by a Danish teamwork (Callesen *et al*., 1987). This study was carried out in seven superovulated heifers. By rectal palpation, the ovaries were positioned against sacrosciatic ligaments and follicles were visualized by ultrasound examination. A total number of 38 follicles were trans-cutaneously punctured and 16 oocytes were collected which resulted in a recovery rate of 42% (RR = number of oocytes collected/number of follicles punctured) and 2.3 oocytes/heifer.

In 1988, *in vivo* oocyte collection by transvaginal ultrasound-guided follicle aspiration (Ovum Pick-Up: OPU) was first established in cattle by a Dutch team (Pieterse *et al*., 1988). These researchers demonstrated that the repeated oocyte collection by OPU could be performed without risks to health and reproductive activity. In this study, OPU was performed once a week in 10 cows for a total number of 36 transvaginal aspiration procedures, during which 54 oocytes were recovered from 197 punctured follicles. The mean RR was 27.4% and the number of oocytes/cow/sampling was 1.5. The stimulation of the ovaries with pregnant mare serum gonadotropin (PMSG) increased both RR (40 *vs.* 18%) and the number of oocytes/cow/sampling (2.7 *vs.* 1.0). The estrous cycle of these animals was not interrupted due to OPU procedure on the basis of plasma progesterone measurements. The oocytes collected were *in vitro* matured and fertilized and then transferred to ligated sheep oviducts resulting in 24% embryo development at morula/blastocyst stages (Kruip *et al*., 1991).

The same team evaluated the effect of repeated once weekly OPU samplings on the estrous cycle of the treated animals (Pieterse *et al*., 1991). The experimental animals were divided in three groups, i.e. A, B1 and B2. Ten cows were submitted to OPU sampling

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once weekly for a 3-month period (A group); in 9 of these cows, OPU sampling was carried out for an additional 3-month period (B1 group); at the same time of B1 group samplings, a new group of 11 cows was submitted to OPU (B2 group). OPU was performed on day 3-4, day 9-10 and day 15-16 of the estrous cycle. The mean estrous cycle length after repeated follicle puncture did not differ among the three groups. The largest number of follicles per puncture session (PS) in all three groups was always on day 3-4 of the estrous cycle (4.9 ± 0.3 *vs.* 3.4 ± 0.2 and 3.9 ± 0.2 follicles at day 3-4 *vs.* 9-10 and 15-16, respectively). Overall, a mean number of 12.6 follicles were punctured and 6.9 oocytes were collected per cow during an estrous cycle by using three PS. By using this technique, however, the number of punctured follicles and collected oocytes were dramatically lower than the mean number of follicles and oocytes present in the ovaries at any given moment of the estrous cycle and collectable in ovaries obtained from slaughtered animals. The reasons for such low number of follicles are undoubtedly due to the low sensitivity of the ultrasound equipment employed, which could visualize only follicles larger than 3 mm. Other technical aspects such as vacuum pressure and needle quality may have affected RR and oocyte quality. In fact, by using the same ultrasound equipment, the increase of vacuum pressure precision (set at 40-50 mmHg) together with a better choice of the needle (18 G, short bevel) improved both OPU efficiency and the quality of the oocytes (Boni *et al.,* 1992).

Another aspect to consider for the optimization of OPU is the frequency of sampling. In fact, once weekly oocyte collection is not able to provide a valuable number of oocytes for IVEP. Conversely, the number of oocytes collected by OPU significantly improved by changing the frequency of collection from once to twice weekly either in PMSG stimulated or in non-stimulated animals (van der Schans *et al*., 1991; Boni *et al*., 1992). Twice weekly OPU schedule resulted in: 1) an increased follicular wave frequency; 2) an arrest of the estrous cycle, follicle maturation and ovulation. Animals submitted to this sampling regimen entered a para-physiological status in which follicular waves were uncoupled from the estrous cycle (Kruip *et al*., 1994). However, as soon as OPU sampling ceased, ovulation took place within 6 days (Boni *et al*., 1993). A further increase in oocyte sampling frequency (PS interval = PSI = 2 day) was not beneficial as it caused a decrease in both the number of punctured follicles (Boni *et al*., 1997) and recovered oocytes (Boni *et al*., 1995), although a higher recovery rate and a better oocyte quality was recorded (Simon *et al*., 1993).

Improvements of OPU technique

Since its establishment, OPU technique underwent permanent attempts for improving the efficiency of either the number or the quality of the collected COCs.

The needle and the aspiration vacuum characteristics play a crucial role in determining the quantity and quality of the collected COCs. Bols *et al*. (1996, 1997) evaluated the effect of these parameters on the morphological quality and the number of the collected COCs by simulating OPU samplings with slaughterhouse ovaries. Comparing three different needle diameters (18, 19 and 21 G) and different vacuum pressures, they obtained the highest oocyte recovery with the thickest needle (18 G) regardless the aspiration vacuum. In addition, for all needle types, more oocytes were recovered at the highest aspiration. On the other hand, the proportion of oocytes surrounded by compact cumulus and *in vitro* produced blastocysts decreased progressively as the vacuum increased. Similar results were also obtained by Ward *et al.* (2000) who recorded a significant decrease of *in vitro* blastocyst production as the aspiration pressure increased beyond 50 mmHg. A good compromise should be established between the collection efficiency and the quality of the COCs. In fact, the mechanical damage generated by the COC transport through the needle depends on the needle size and length as well as the vacuum pressure. However, while the former two parameters are chosen by the operator, the latter depends on needle size and slope as well as on other factors, such as the opposite pressure present in the vagina and the peritoneum. These characteristics impair the evaluation of the best vacuum pressure by using simulation experiments with slaughterhouse ovaries and forces to adapt vacuum pressure under operative conditions.

Also the tip bevel and the sharpness of the needle affect the oocyte recovery. Bols *et al*. (1997) demonstrated a better RR when long bevelled needles were used. The discrepancy between long and short bevel, however, increased together with vacuum pressure. Other authors, as Fayrer-Hosken and Caudle (1991) and Kruip *et al*. (1994), on the contrary, chose short bevel needles on the basis of conceptual or unpublished considerations. The effect of the sharpness of the needle can be easily evaluated under operative conditions; since the re-use of needles decreases their sharpness, attempts were made to make the needle substitution easier and cheaper by using disposable needles. Special 55 cm long needles are now commercially available; they are totally replaced after 3-4 PS or the replacement involves just the tip that is fixed by glue on a 17 G single lumen needle (Kruip *et al*., 1994; Galli *et al*., 2001). Bols *et al*. (1995) proposed an OPU device that mounted 19 G disposable needles that were connected to silicone tubing by means of a stainless steel connector. This system was inserted into a stainless steel tube, creating a rigid structure that allowed the needle to move back and forth. Also Bungartz *et al*. (1995) evaluated the use of disposable

needles that were connected to a permanent rinse tubing system; this procedure, however, did not obtain a commercial success maybe due to the large amount of flushing fluids that were necessary to rinse or wash the large catheter at which the disposable needles were grafted. A further increase of oocyte recovery can be obtained by twisting the needle within the follicle (Fayrer-Hosken and Caudle, 1991). This technique showed a significant improvement of approximately 30% of the RR (Sasamoto *et al*., 2003) due to a better detachment of the COC by curettage of the follicular wall during the follicle aspiration.

The sensitivity of the ultrasound equipment represents another parameter of extreme relevance for the OPU efficiency. The first study on OPU in The Netherlands used a 5.0 MHz sector scanner probe allowing the visualization of follicles larger than 3 mm. The passage to a 6.5 MHz curved array probe significantly improved either the number of visible follicles or the number of the collected oocytes (Kruip *et al.,* 1994). A comparison between a linear array and a mechanical multiple angle sector (MAP) transducer for OPU was made by Bols *et al*. (2004). The ovaries of 5 dairy cows were punctured, in a twice-weekly OPU program lasting for 4 weeks, using two different 5.0-MHz transducers equipped with an identical disposable needle-guidance system. Both ovaries were visualized using each transducer before puncture and the number of follicles with a diameter <5 mm (small) and with a diameter ≥5 mm (large) was recorded. Subsequently, one ovary of the pair was punctured guided by the MAP, while the other was punctured using the linear array transducer. During the next puncture session on a given animal, the two systems were switched and used on the alternate ovary in a crossover design. A significant difference was found for the ability to visualize smaller follicles in favour of the MAP transducer, with an average visualization of 71.6 ± 30.3 small follicles per cow during the 4-week trial period, compared to 59.8 ± 25.7 for the linear array transducer. No differences were found in the visualization of large follicles. A greater number of oocytes were retrieved using the MAP transducer, compared to the linear array (14.2 ± 7.2 *vs.* 7.4 ± 6.1, respectively).

Another possibility to collect oocytes from living cows was proposed by Reichenbach *et al*. (1994) who developed a laparoscopic procedure of oocyte collection (L-OPU). This technique allows the repeated laparoscopic examination of the internal reproductive organs of cows and heifers through the vaginal fornix and visually assisted follicle aspiration. It can be performed in a simple crush in less than 15 min, does not require surgery and can be used under field conditions. The method has been used for aspirating oocytes from follicles that were at least 2 mm in diameter in animals under sedation and epidural anaesthesia. In this study, 11 cows and 8 heifers were allocated into two groups: 12 animals were treated weekly with 500 IU PMSG and 7 animals were not stimulated. Mean numbers of oocytes collected from the treated cows and heifers (6.3 and 3.3) did not differ significantly from numbers collected from non-stimulated cows and heifers (5.5 and 4.0). When donors were aspirated twice instead of once weekly, the mean number of follicles observed (16.2 *vs.* 7.0) and the mean number of oocytes collected per week (12.2 *vs.* 5.2) resulted significantly higher ($P < 0.05$). L-OPU showed several advantages with respect to OPU; in particular, i) the aspiration of primarily superficial follicles; ii) the direct view of the ovary and the aspiration procedure; iii) a reduced risk of injury to the ovary. L-OPU and OPU techniques were compared by Becker *et al*. (1996) and Santl *et al*. (1998). From these studies, OPU allowed to collect a significantly higher proportion of grade-1 oocytes that in turn lead to higher cleavage and morula-blastocyst rates than L-OPU.

OPU for *in vitro* embryo production

Kruip *et al*. (1994) released the first detailed report of OPU application twice weekly over a long period in cattle. Three kinds of experiments were conducted to examine the effects of puncturing on follicle recruitment and number of oocytes collected at regular intervals of 3-4 day. The oocytes were *in vitro* matured and fertilized and the number of transferable embryos was recorded. In Experiment 1, dairy cows (n = 10) were punctured over a period of 5 months, and the collected oocytes were fertilized with the semen of one bull. In Experiment 2, oocytes were collected from one 12-yr old high pedigree dairy cow and one 1-month pregnant cow. In Experiment 3, beef cows (n = 6) were punctured over a 2-month period and the semen of 2 different bulls of the same breed was used to fertilize the oocytes from 3 cows. In Experiment 1, 14.5 ± 0.4 follicles were punctured per session, and 8.0 ± 0.3 oocytes were recovered. A mean of 16% of the oocytes developed into blastocysts with a pregnancy rate of 40%. The results did not differ between the months of the experiments, indicating that the transvaginal puncturing method can be used successfully over a 5-month period; thus, a hypothetical number of 340 collected oocytes and 54 transferable embryos per cow over the 5-month period can be calculated. No detrimental effects were observed after clinical and post-mortem examinations, nor did breed, age or reproductive status appear to affect the results. However, large differences were observed between individual cows and between cow/bull combinations (Boni *et al*., 1995). In particular, the use of the semen of 2 bulls to fertilize the repeatedly collected oocytes from 3 Blond d'Aquitane cows highlighted either a male effect, which was signalled by the different percentages of blastocysts provided by the 2 bulls ($P < 0.01$) or a female effect, which was signalled by the different IVEP efficiency between females within bulls. The

possible maternal influence on IVEP in cows has been more deeply analyzed by Tamassia *et al*. (2003) who demonstrated that, although oocyte production and embryo production are independent factors, the oocyte donor influences the production of blastocysts.

Up to date, OPU technique did not show significant improvements in terms of recovered oocytes; however, the number of transferable embryos provided by OPU is significantly increased due to the optimization of IVEP technology. Several parameters have been evaluated in order to improve the efficiency of OPU and make this technique more and more competitive to superovulation for embryo production in cattle.

Since the OPU technique was established, the frequency of sampling represents a crucial point. The passage from once to twice weekly OPU sampling coincided with a significant increase of the OPU efficiency (van der Schans *et al*., 1991; Boni *et al*., 1992). Detailed comparisons of these two sampling regimens have been deeply evaluated (Gibbons *et al.*, 1994; Lopes *et al*., 2006; Li *et al*., 2007) and there is a general agreement that twice-weekly aspiration yields a higher number of viable oocytes and transferable embryos than once-weekly aspiration. Recently, using different OPU sampling schemes, i.e. 1) twice a week (every 3 or 4 days); 2) every 5 days; 3) once a week (every 7 days); 4) every 10 days; and 5) once every 2 weeks (every 14 days) for the collection of oocytes used for embryo cloning, Ding *et al*. (2008) obtained the following rates of development of the reconstituted embryos to the blastocyst stage: 23.1% (Group 1), 15.0% (Group 2), 10.9% (Group 3), 4.9% (Group 4), and 29.0% (Group 5). These results indicate that the developmental potential of follicles from the same living donors are different when different intervals of OPU samplings are adopted; this may be due to the positive relationship between the PSI and the atresia grade of the follicle population (Boni *et al*., 1996).

Another aspect of the OPU efficiency is related to its continuity. Petyim *et al.* (2003) compared the twice-weekly OPU application by using continuous or discontinuous (i.e. restricted to days 0-12 of the estrous cycle) schemes. The mean number of punctured follicles and collected oocytes as well as the oocyte quality and the cleavage rate did not differ per PS between the two OPU schemes. The discontinuous OPU scheme permits a normal ovulation and corpus luteum (CL) formation that shows characteristics similar to those of the pre-OPU period. Heifers submitted to continuous OPU scheme barely showed cyclicity with irregular inter-estrous intervals and weaker signs of estrous.

Many attempts of hormonal stimulation have been evaluated in order to improve follicle population in animals submitted to OPU. Pieterse *et al*. (1988) treated cows with 1000-3000 IU PMSG in the presence of an active mid-cycle CL 2 days before OPU. The mean number of aspirated follicles significantly increased in stimulated compared to non-stimulated donors. On the other hand, the treatment decreased the RR value. In problem donor cows that had not been successful in conventional embryo production, Looney *et al.* (1994) collected an average of 6.3 oocytes per aspiration by using once a week schedule. The stimulation with 4-5 mg pFSH for 3 days prior to oocyte retrieval increased the oocyte retrieval and the number of grade 1–2 embryos per session compared to non-treated animals (1.38 *vs.* 0.96). In pregnant donors either treated with 20 or 40 mg FSH or non-stimulated, Meintjens *et al.* (1995) found highest percentage of viable oocytes in cows that received 40 mg FSH. Stubbings and Walton (1995) did not detect any differences in the mean number of follicles available per PS between non-stimulated cows punctured twice weekly and FSH-stimulated cows punctured once weekly. Bungartz *et al*. (1995) compared the OPU efficiency in 8 cows that were or were not stimulated 4-day prior to OPU aspiration with a single injection of 100 mg pFSH. The number of aspirated follicles was higher in the treated group (10.6 ± 0.7 *vs*. 8.9 ± 0.5; $P < 0.05$); however, the number of collected oocytes (7.0 ± 0.6 *vs.* 5.8 ± 0.5), RR (66.6% *vs.* 65.4%), percentage of viable oocytes (56.8% *vs.* 52.1%), cleavage (56.7% *vs.* 59.8%) and morula/blastocyst (3.8% *vs.* 2.9%) rates did not differ between treated and non-treated groups. Blondin *et al.* (2002) proposed a superstimulation protocol in which multiple injections of FSH and a single LH injection (at 6 h before OPU) were administered. Collecting oocytes that had initiated the maturation process largely increased IVEP efficiency.

Chaubal *et al.* (2006) evaluated the effects of once *vs.* twice-weekly OPU, dominant follicle removal (DFR) and FSH stimulation (once weekly 200 mg Folltropin divided in 80 mg IM and 120 mg SC) prior OPU on five groups of 3 cows each; each group was allotted to a treatment protocol, which was repeated every week for 10 consecutive weeks. Treatment with FSH, followed by twice-weekly OPU, failed to show any synergistic effect of FSH and increased aspiration frequency. When FSH was given 36 h after dominant follicle removal (DFR), followed by OPU 48 h later, more ($P < 0.05$) follicles, oocytes and embryos were obtained during each session, but not on a weekly basis. Pooled results over 10 weeks showed an overall improved performance for the treatment groups with twice-weekly OPU sessions, due to the doubled number of OPU sessions performed. However, the protocol that consisted of DFR, FSH treatment and a subsequent single OPU per week was considered the most productive and cost-effective. In a large scale retrospective study in beef cattle, De Roover *et al*. (2008) compared twice weekly OPU application in non-stimulated cows to once every 2 weeks OPU application in cows which were treated as follows: i) 8 days before OPU with the insertion of an ear implant containing 3 mg norgestomet (Crestar, Intervet, Belgium); ii) the dominant follicle was ablated by ultrasound-guided follicle puncture on day 6; iii) on -3 and -2 day, cows

were injected with FSH (Ovagen, ICP) twice daily, i.e. a total dose of 160 µg FSH per donor per stimulation cycle. Animals were punctured 48 h after the last FSH injection (day 0). Stimulated donor cows were treated with this protocol at 14-day intervals. FSH stimulation prior to OPU increased production efficiency with significantly more follicles punctured and oocytes retrieved. However, when overall results during comparable 2-week period were considered (four non-stimulated sessions *vs.* one stimulated), more follicles were punctured and more oocytes were retrieved using the non-stimulated protocol. The absence of significant differences between these two regimens in the number of *in vitro* produced embryos within the 2-week period suggests a positive effect on *in vitro* oocyte developmental competence in the treated animals.

The OPU efficiency did not show a significant variation among cow breeds as long as *Bos taurus* breeds are concerned (Kruip *et al*., 1994). If this evaluation is extended to *Bos indicus* breeds and *indicus-taurus* donors significant differences were observed. Pontes *et al.* (2010) reported a large-scale commercial program for *in vitro* production of embryos from dairy *Bos taurus*, *Bos indicus*, and *indicus-taurus* donors, using sexed sperm. The number of viable oocytes per OPU session was 12.1 ± 3.9 for Gir cows, 8.0 ± 2.7 for Holstein cows, 16.8 ± 5.0 for 1/4 Holstein x 3/4 Gir, and 24.3 ± 4.7 for 1/2 Holstein-Gir crossbred females ($P < 0.01$). The mean number of embryos produced by OPU-IVEP and the pregnancy rates were 3.2 and 40% for Gir cows, 2.1 and 36% for Holstein cows, 3.9 and 37% for 1/4 Holstein x 3/4 Gir, and 5.5 and 37% for 1/2 Holstein-Gir.

Brilliant results come from OPU application in beef *Bos indicus* breeds. A very recent report by Pontes *et al.* (2011) reported data from 656 OPU+IVEP procedures performed on 317 Nelore donors, without hormone stimulation or control of ovarian follicular waves. Donors were subjected to OPU from one to nine times, with <15 days between consecutive procedures. The mean (± SEM) number of total and viable oocytes produced per OPU session was 30.8 ± 0.9 and 23.4 ± 0.7, which yielded 8.1 ± 0.3 embryos and 3.0 ± 0.1 pregnancies per OPU-IVEP procedure (pregnancy rate at the 60th day = 33%). A very large variability had been observed among individual cows with a range from 0 to 128 oocytes. There was no significant effect of the number of OPU sessions per donor on mean numbers of oocytes produced.

OPU can be successfully applied in pregnant cows (Kruip *et al.,* 1994). Recently, Takuma *et al.* (2010) compared the OPU efficiency in pregnant and empty cows between hot and cool seasons. The quality of the collected COCs did not differ in relation to the reproductive phase of the donor cows. Analyzing the two reproductive phases, however, the number of follicles and collected oocytes significant decreased during the hot season, limitedly in empty cows. In addition, when data from the two seasons were combined, pregnant cows showed a significant higher proportion of cleavage and blastocyst rates and freezable embryos than empty cows.

OPU can be applied in early post-partum cows without negative effects on the number and quality of the retrieved oocytes (Lopes, 2006). Aller *et al.* (2010) evaluated the overall efficiency of the OPU-IVEP from days 30 to 80 postpartum in FSH-stimulated and non-stimulated cows and the fertility of these cows following a fixed-time artificial insemination (FTAI) protocol. The number of punctured follicles and recovered oocytes was larger in treated cows. However, cleavage and embryo development rates were similar for both groups. After FTAI, pregnancy rates were independent of OPU treatment.

OPU for research purpose

OPU can be used as a tool for interfering with follicle dynamics in order to advance our knowledge in its regulatory mechanisms and for technology purposes.

The continuous application of OPU twice weekly doubles the frequency of follicular waves, which becomes uncoupled from the estrous cycle because of inhibition of ovulation. In addition, the absence of a CL avoids interference of the progesterone on follicle growth. This particular condition together with the removal of each follicle larger than 2 mm makes easier to study either the growth or the replacement of follicles. Using this experimental approach, we analyzed the follicle growth in 10 cows submitted to a continuous twice-weekly OPU sampling for a 3-month period and, limitedly in 4 of these cows, for an additional 3-month period (Boni *et al.,* 1997). The mean ± SD number of recruited follicles varied between animals from 7.8 ± 2.5 to 19.2 ± 6.0. A progressive increase of recruited follicles occurred from the first (March) to the third (May) month of sampling (11.8 ± 4.7 *vs.* 16.4 ± 6.5; $P < 0.01$); after that, a slight decrease was observed. The repeatability of the follicle growth showed a high value ($r = 0.576$), and a good predictability of the potential of the animal to recruit follicles was observed on the first four to six PS. Since the repeatability indicates the upper limit of the heritability, further studies are auspicated to identify the genetic basis for this feature that may represent a key-point for future breeding programmes. In support of this hypothesis, Machado *et al*. (2006) have demonstrated a significant larger variability of total number of collected COCs and *in vitro* produced blastocysts among pairs of monozygotic twins than within pairs. Recently, genetic parameters related to reproduction were analyzed by using data sets from eleven years of OPU-IVEP application over 1508 HF donors and 18.702 OPU by Holland Genetics (Merton *et al.,* 2009). This study estimated that heritability of the number of COCs collected by OPU was 0.25. Since there is a good correlation between the number of

follicles punctured and the number of oocytes collected by OPU ($r = 0.742$, $P < 0.0001$; Boni and Kruip, unpublished), we may argue that also the heritability of follicle recruitment may reach a similar value. If this hypothesis is confirmed, the genetic selection for follicle recruitment might be introduced in breeding programs for enhancing the reproductive performance of future herds.

OPU has been proposed as a tool for studying luteal function. A study in *Bos indicus* and crossbred cows, which were submitted to a daily OPU sampling of follicles >6 mm in diameter from day 13 to day 25 of the estrous cycle, evaluated the possibility of a relationship between follicle growth and steroidogenesis in the modulation of luteal function (Bisinotto *et al.,* 2012). In spite of an increase of the estrous cycle length and a decrease of the follicle diameter, OPU failed to delay luteolysis.

The relationship between ovarian follicular dynamics and the number and quality of the recovered COCs was evaluated in Zebu cows by Viana *et al.* (2010). These authors found that follicular dominance could be established in cows undergoing twice-weekly OPU, and that the presence of a dominant follicle did not affect COC quality, except when a co-dominant follicle was present.

Heat stress has been evaluated on follicle growth and oocyte competence by using OPU in *Bos indicus* cattle (Torres-Júnior *et al.,* 2008). Exposure to heat stress had no immediate impact on reproductive function, but exerted a delayed deleterious effect on ovarian follicular growth, hormone concentrations, and oocyte competence. Following a 28 day experimental period of heat stress, cows had longer periods of non-cyclic activity, as well as shorter estrous cycles; the diameter of the dominant and double-dominant follicles as well as the number of follicles >9 mm in diameter significantly increased; IVEP efficiency of collected oocytes did not vary at the time of high temperature and humidity exposure, but it significantly decreased in the following period of thermoneutrality.

OPU can be used to eliminate the deleterious effect of the presence of a dominant follicle during superovulation in cows. By puncturing the dominant follicle 38-46 h prior to superovulatory treatment, Holland Genetics practitioners improved the mean number of transferable embryos in cows (5.4 ± 0.5 *vs.* 3.9 ± 0.4; $P < 0.05$) rather than in heifers (4.3 ± 0.6 *vs.* 4.0 ± 0.4; Merton *et al.,* 2003).

The OPU application efficiently resets the >2 mm diameter follicular population to zero which in effect guarantees that the new population will be uniformly renewed with less atresia (Boni *et al*., 1995). We used this approach in order to manipulate follicle population of buffalo cows submitted to superovulation (Zicarelli *et al*., 1995). By performing OPU sampling 24 h prior to superovulatory treatment, we have found an improved efficiency of superovulatory response in some periods of the year and no significant differences in other ones. Further evaluation of this approach may be investigated in cattle by using a different interval between OPU and superovulatory treatment onset.

Finally, special modifications of OPU have been used to collect ovarian tissue for primary follicle isolation (Aerts *et al.,* 2005). A reverse application of the technique established for OPU was proposed by Bergfelt *et al*. (1998). These authors transferred 6-7 COCs within the preovulatory follicle (Gamete Recovery And Follicular Transfer = GRAFT) of synchronized recipient heifers (n = 7), by using a transvaginal ultrasound-guided follicular puncture, in order to take advantage of follicle and oviduct environments for *in vivo* maturation and fertilization. A total number of 8 oocytes and 8 embryos were collected from the oviducts of five out of the seven heifers.

Conclusions

OPU has been greatly spread over the years due to the increased number of transferable embryos, mainly due to the improvements of the *in vitro* embryo production technologies. Limits to OPU application are still represented by lower pregnancy rate of *in vitro vs. in vivo* produced embryos and quality of cooperation between OPU practitioners and IVF laboratories.

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