LAPAROSCOPIC OVUM PICK-UP (LOPU): FROM ANIMAL PRODUCTION TO CONSERVATION

Aspiración folicular por Laparoscopia (LOPU): de la producción a la conservación

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ABSTRACT

Laparoscopic Ovum Pick-Up (LOPU) is the most reliable and efficient technique for collecting high quality oocytes from live animals in certain species or age groups, allowing its use for In Vitro Embryo Production (IVEP) and Somatic Cell Nuclear Transfer (SCNT). In order to maximize the number and quality of oocytes collected by donor, it is necessary to synchronize estrus and stimulate follicular growth using hormonal protocols that vary according to species. There are 2 big categories of applications for the LOPU-IVEP technology in production animals, those in which it acts as an alternative to MOET (competitive applications) and those in which it doesn’t compete with MOET as it cannot be done in those categories (non competitive applications). In wild animals, LOPU can play an important role in conservation programs for endangered species when associated with effective IVEP and has been done in several species. It has commercial application in sheep, goats, cattle and buffaloes calves. Repeating LOPU procedures in the same female does not cause sequels with impact on the female’s reproductive life, even when performed on prepubertal or wild animals.

Keywords: Laparoscopic Ovum Pick-up, prepuberal, sheep, goat, cattle, wild animals

RESUMEN

La recuperación de ovocitos por laparoscopia (LOPU) es la técnica más confiable y eficiente para recolectar ovocitos de alta calidad de animales vivos en ciertas especies o grupos de edad, lo que permite su uso para producción de embriones in vitro (IVEP) y transferencia nuclear de células somáticas (SCNT). Asimismo, para maximizar la cantidad y calidad de los ovocitos recuperados por el donante, es necesario sincronizar el estro y estimular el crecimiento folicular mediante protocolos hormonales que varían según la especie. Hay dos grandes categorías de aplicaciones para la tecnología LOPU-IVEP en animales de producción, aquellas en las que actúa como una alternativa a MOET (aplicaciones competitivas) y aquellas en las que no compite con MOET ya que no se puede hacer en esas categorías (aplicaciones no competitivas). En animales silvestres, LOPU puede jugar un papel importante en los programas de conservación de especies en peligro cuando se asocia con un IVEP efectivo y se ha realizado en varias especies. Tiene aplicación comercial en ovinos, caprinos, bovinos y búfalos. La repetición de los procedimientos LOPU en la misma hembra no causa secuelas con impacto en la vida reproductiva de la hembra, incluso cuando se realiza en animales prepúberes o salvajes.

Palabras clave: Recolección de ovocitos por laparoscopia, prepuberal, ovino, caprino, vacuno, animales salvajes.
INTRODUCTION

Reproductive biotechnologies are in constant development, and In Vitro Embryo Production (IVEP) has become an important tool for improving the genetic merit of cattle, buffaloes, sheep and goats, as well as a technology with enormous potential for the conservation of wild animals.

Laparoscopic Ovum Pick-Up (LOPU) is the most reliable and efficient technique for collecting high quality oocytes from live animals in certain species or age groups, allowing its use in IVEP and Somatic Cell Nuclear Transfer (SCNT).

The technique is minimally invasive and has repeatability and reliability, allowing the observation of the ovaries through the laparoscope and the puncture of the follicles, besides a fast recovery of the female after the procedure and in general, without surgical complications. In contrast to Multiple Ovulation and Embryo Transfer (MOET), LOPU brings the advantages of being less invasive and causing less postoperative stress, and can be repeated in a shorter interval (minimum of 60 days for MOET against a minimum of 14 days for LOPU), besides causing no adhesions or fibrosis and adhesions commonly occurring after multiple MOETs.

LOPU also allows the year-round production of embryos, even outside the breeding season.

OVARIAN SUPERSTIMULATION

In order to maximize the number and quality of oocytes collected by donor, it is necessary to synchronize estrus and stimulate follicular growth prior to LOPU, through protocols based on gonadotropins, which exert a significant influence on the results.

Synchronization is performed using intravaginal devices containing prostaglandine or progestogen (eg, CIDR, Primer, sponges) which are applied with a luteolytic dose of prostaglandin or analog (eg, cloprostenol, dinoprost) when treatment with gonadotropins initiates.

Several treatments have been proposed for the stimulation of follicular growth, being the two most popular the protocol with multiple FSH injections and the Oneshot protocol. The first one consists of 3 FSH injections of 1.5, 1.5 and 1 mL, totaling 80 mg FSH (Folltropin-V, Vetoquinol N.-A inc, Canada) administered at 12-hour intervals and starting 36 hours before LOPU. The second consists on the application of 4 mL of FSH together with 300 IU of eCG (Folligon, Intervet, The Netherlands) and no further injections until LOPU (total of 120 mg FSH in 5 injections). No statistical differences were observed between the 3 treatments in terms of average follicles available for aspiration (35.7 ± 16 vs. 38.5 ± 25 vs. 31.1 ± 22), mean oocytes recovered 21.6 ± 10 vs. 19.4 ± 14) and cleavage rate (66.0 ± 14 vs. 61.1 ± 11 vs. 72.2 ± 8) for FSH12h, FSH8h and FSH12h-eCG, respectively. However, FSH8h-eCG resulted in a significantly higher rate of transferable embryos (17.5 ± 8%) compared to FSH12h (8.9 ± 5%, P <0.05).

In wild animals the protocols have great variations due to the particular physiology of each species. In animals studies treated team with cougars (Puma concolor) and jaguars (Panthera onca), in order to prepare the females for LOPU, 750 IU of eCG were used to stimulate multiple follicular development 5 days before the procedure. In some donors, 500 IU of hCG were also used to try to mature the oocytes in vivo before LOPU, but the results obtained were very variable (Baldassarre et al. 2017b and unpublished results). In gazelle dama (Gazella dama mhorr), Berlinguer et al (2008) synchronized estrus with intravaginal progesterone device (CIDR) for 15 days. CIDRs were replaced on day 10 and removed during LOPU on day 15. Follicular growth was stimulated by administration of a total of 5.28 mg ovine FSH (Ovagen, IPCbio Ltd, New Zealand) applied in four equal doses given at 8:00 p.m. and 6:00 p.m. on days 13 and 14.

In red deer (Cervus elaphus), Bainbridge et al. (1999) used the same CIDRs for 10 days, applying 1,000 IU of eCG 48 h prior to removal and performed LOPU 24 h after. Locateili et al. (2006) performed weekly LOPUs on sika dear (Cervus nippon nippon), synchronizing estrus intravaginal sponges (45 mg FGA, Intervet, France) during the breeding season and stimulated the ovaries with three injections of FSH of 0.2, 0.2 and 0.1 IU, at 12 hour intervals, starting 48 h before LOPU.

Description of the LOPU Technique

The equipment used for LOPU consists of a laparoscope (diameter of 5 or 7mm, angle of 0° or 30° and length of ~30cm) connected to a cable and light source. One pump of insufflation, three trocars (two of 5mm being one with valve for insufflation and one of 3,5mm) and anatraumatic endoscopic
forcepts. The suction system is composed of a suction pump connected to the OPU stopper connected to a 50 ml Falcon-type tube and connected to the LOPU pipette. The pressure of the vacuum pump should be measured in drops per minute (velocity of the aspiration medium through the aspiration pipette and falling into the collection tube) and adjusted to 50-70 drops min (Baldassarre et al., 1994), the system must be washed with aspiration medium before and after LOPU.

The LOPU technique was described by Baldassarre et al. (1994). Animals should be fasted for 12 (water) to 24 hours (solids) before LOPU. The anesthetic protocol varies according to the species, consisting of a sedative to position the animal on a cradle typically used for laparoscopy insinimation and then is then connected to the anesthesia machine and maintained under general anesthesia with isoflurane. A tricotomy is performed in the abdominal region followed by disinfection of the area cranial of the mammary gland. Once the female reaches an anesthetic plane, the cradle is tilted to Trendelenburg position (variation of the dorsal decubitus position where the upper back is lowered and the legs are elevated, keeping the intestinal loops in the upper abdominal cavity at 45 degrees. Three small incisions are made with the aid of a scalpel blade for the easy entrance of the trocars, and the location of the incisions will vary according to the species. The first trocar (with insufflation valve) must be introduced with counter-traction for abdominal elevation, avoiding sudden movements or excessive force ( Campos & Roll, 2003). Then, filtered air is blown into the abdominal cavity to separate organs and facilitate visualization. In sequence, the other two trocars are inserted, under direct visualization by the laparoscope. The ovary is then exposed for visualization and puncture of the follicles, moving the fimbria in different directions with the endoscopic forceps. The aspiration needle should be entered by the side of the follicle, avoiding vascularized areas, parallel to the base of the follicle. If this is not possible, the puncture occurs perpendicular to the follicle wall (Baldassarre et al., 1994). At the end of follicular aspiration, both ovaries are washed with 0.9% sodium chloride (physiological solution) to eliminate any clots formed by the follicular puncture, avoiding adhesions. The trocars are then removed and the incisions are glued with instant adhesive or sutured, depending on the species. They are given antibiotic and anti-inflammatory and the female is withdrawn from the anesthetic plane, recovering in a calm and safe place. The collection tube is passed to the lab for finding and grading of the oocytes under the stereomicroscope.

LOPU in Farm Animals

LOPU presents exceptional performance in specific applications in production animals, allowing to increase the production of offspring from females of high genetic merit and also the production of progeny from categories that are not eligible for MOET (Baldassarre et al., 2004). According to Tervit (1996), the technology has been applied to species or age groups where it is not possible or easy to manipulate the reproductive tract through the rectum during oocyte retrieval.

There are 2 big categories of applications for the LOPU-IVP technology in production animals, those in which it acts as an alternative to MOET (competitive applications) and those in which it doesn’t compete with MOET as MOET cannot be done in those categories (non-competitive applications).

COMPETITIVE APPLICATIONS

This application refers to the use of non-gravid, non-lactating/weaned, adult females as donor of oocytes for LOPU-IVP. Used in this category, LOPU-IVP is for the exact same objective as MOET, i.e. production of more progeny from outstanding adult females. In a 1:1 comparison, MOET should win this competition because in normal conditions MOET should result in an average of 8-10 transferable embryos/flush while LOPU-IVP will produce an average of 4.5 blastocysts for transfer, and the viability of in vivo produced embryo tends to be better than the in vitro counterparts. So, in this specific application, the advantage of using LOPU-IVP applies only to customers willing to produce many more progenies from their top females, that what can be achieved by 1-2 MOET flushes per year. In those cases, LOPU-IVP offers the option of oocyte collection every 2 weeks almost unlimitedly (30 times in the same animals in 4 years, Baldassarre et al. personal communication). For example, 3 MOET in one year can result in 24-30 transferable embryos, but in the same period one can easy conduct 1 LOPU every 2 weeks and end with 48-60 transferable embryos for transfer. And, 3 surgical embryo collections has a high chance of developing adhesions, while 12 LOPU will produce no sequels in the donors.

NON-COMPETITIVE APPLICATIONS:

This refers to the categories where MOET doesn’t work so it is not a reproductive technology that can be applied for exponential multiplication of valuable females. Some of these categories are age-related, specifically the use of LOPU-IVP for early reproduction of elite females before reaching puberty a sexual maturity; as well as older animals that have become unfruitful with age or incapable of carrying a pregnancy themselves but are genetically superior. Other non-competitive categories refer to animal “conditions”, most of them temporary conditions that prevent the animals from producing embryos by MOET for a period, such as early pregnant animals and early post partum animals. Finally, another category of non-competitive application refers to the practice of LOPU-IVP in MOET failures, specifically animals with history of repeated failure. These are animals that have been subjected to MOET programs repeatedly and they have always failed to produce transferable embryos for reason of a) repeated luteal regression; b) failure to ovulate; c) failure to terminate (only unfertile eggs are recovered). Finally, LOPU-IVP offers opportunities for reproductive rescue of females that have been “crippled” by previous surgeries ending in a uterus with so many adhesions that they can’t be flushed any more, maybe even not get pregnant any more. As long as their ovaries are clean, oocytes can be collected and embryos produced in vitro to continue propagating their outstanding genetics.

The prepubertal females of the ovine, caprine, buffalo and bovine species between two and seven months of life have a greater response to gonadotrophins in relation to adult animals, allowing to produce embryos of females with only two reproductive cycles per year. In those cases, LOPU-IVP is better than the in vitro counterparts. So, in this category, LOPU-IVP will result in an average of 8-10 transferable embryos/flush while IVEP will produce an average of 4.5 blastocysts for transfer, and the viability of in vivo produced embryo tends to be better than the in vitro counterparts. So, in this specific application, the advantage of using LOPU-IVP applies only to customers willing to produce many more progenies from their top females, that what can be achieved by 1-2 MOET flushes per year. In those cases, LOPU-IVP offers the option of oocyte collection every 2 weeks almost unlimitedly (30 times in the same animals in 4 years, Baldassarre et al. personal communication). For example, 3 MOET in one year can result in 24-30 transferable embryos, but in the same period one can easy conduct 1 LOPU every 2 weeks and end with 48-60 transferable embryos for transfer. And, 3 surgical embryo collections has a high chance of developing adhesions, while 12 LOPU will produce no sequels in the donors.
the application of this technology in prepubertal goats results in the birth of their progeny at about the same time that donors reach the age and weight for first breeding, and highlight the reduction in the interval of generation that can be achieved. Genetic merit senile females who generally present a high risk of MOET failure due to reasons of low response to superovulation protocol, regression of corpus luteum or infertile embryos, are generally successful in the production of offspring with the use of LOPU-IVEP, making it the tool of choice for obtaining viable embryos of this category (Baldassarre et al., 2007).

LOPU also allows oocytes to be obtained from pregnant females throughout early pregnancy. In postpartum females, it allows the recovery of oocytes before uterine involution, which occurs between the third and fourth week postpartum and consequently, are in this period unfit for MOET.

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Table 2. Results of LOPU at farm animals. Total of LOPU procedures (N-LOPU), Total Aspirated Follicles (TAF), Total Recovered Oocytes (COC). Mean Recovered Oocytes (A-COC) and Average of Oocytes Recovered per LOPU (M-LOPU).

| Species | Category | N-LOPU | TAF  | COC  | M-LOPU | Author |
|---------|----------|--------|------|------|--------|--------|
| Goats   | Adults   | 21     | 399  | 334  | 15.9   | Koeman et al., 2003 |
|         | Adults   | 18     | 221  | 146  | 8.1    | Mendes et al., 2018 |
|         | 2-3 mo.  | 20     | 1.186| 994  | 49.7   | Baldassarre et al., 2002 |
|         | 3-5 mo.  | 36     | 1.238| 987  | 27.4   | Baldassarre et al., 2002 |
|         | 3-5 mo.  | 23     | 897  | 653  | 28.4   | Koeman et al., 2003 |
|         | Senil    | 4      | 772  | 676  | 13.4   | Baldassarre et al., 2007 |
|         | Various  | 1.580  | ~26.523| 21.219| 13.4   | Baldassarre et al., 2004 |
| Sheep   | Adults   | 142    | 1.941| 1.522| 10.7   | Baldassarre et al., 1996 |
| Cattle  | 2-6 mo.  | 63     | ~1.953| 1.351| 21.4   | Baldassarre et al., 2018 |
|         | 2-6 mo.  | 24     | N/A  | 111  | 4.6    | Armstrong et al., 1992 |
|         | 2-4 mo.  | 20     | 1.025| 853  | 42.6   | Taneja et al., 2000 |
|         | Buffalo  | 47     | 903  | 774  | 16.5   | Baldassarre et al., 2017a |

LOPU in the Conservation of Wild Animals

Assisted Reproduction Technologies (ART) can be applied to species conservation. Among the ARTs, LOPU has an important role in endangered species, allowing the efficient recovery of oocytes for use in IVEP and SCNT, tools to reconstruct the balance in the number of animals for these endangered species. Such technologies are useful in rare and endangered animals, as well as species that have difficulty breeding in captivity. They also allow the exchange of genetic material between captive and free-living animals, increasing genetic variability in captivity and restoring free populations with a high degree of inbreeding.

Tervit (1996) points out that the technique may play an important role in endangered species when associated with effective PIVE and it is anticipated that the application of LOPU will increase as biological differences between species are understood.

Baldassarre et al. (2017b) demonstrated that LOPU is a safe procedure for oocyte collection in wild animals, reporting fertility after LOPU is repeatedly conducted, validating the procedure as safe for wild feline multiplication as part of conservation strategies, specifically cougars and jaguars which are considered vulnerable in Brazil. Some other studies were carried out by the authors on wild animals in Brazil and by Baldassarre in the United States and South Africa. Results of oocytes were reported in table 3, to the best of our knowledge, the record oocyte harvest from a live donor by LOPU in wild animals, is 106 oocytes obtained by Requena et al. in a Puma concolor. Also, LOPU was successfully performed on at least seventeen other wild species (table 3).

All those consulted, with several species, demonstrated that LOPU can be applied repeatedly to the same female with no or few adverse effects. As a consequence, the application of the technology to conservation projects only further development or optimization of methodologies for the production of in vitro embryos from the oocytes collected by LOPU.

Table 1. Total recovered oocytes (T-COC). Mean recovered oocytes (M-COC) and maximum number of recovered oocytes from a single donor (MAX) in adult and elderly donors in commercial LOPU services between 2016 and 2017 (Alecho Requena et al., Unpublished).

| Breed / Specie | No. LOPU | T-COC | M-COC | MAX |
|----------------|----------|-------|-------|-----|
| Alpino / Goat  | 11       | 191   | 17.36 | 36  |
| Anglo / Goat   | 6        | 145   | 24.16 | 73  |
| Saanen / Goat  | 38       | 570   | 15.00 | 37  |
| Dorper / Sheep | 58       | 641   | 11.05 | 24  |
| White Dorper / Sheep | 42 | 496 | 11.80 | 27  |
Table 3. Total recovered oocytes (COC). Mean recovered oocytes (A-COC) and Maximum number of recovered oocytes from a single donor (MAX).

| Species                          | No. | LOPU | COC | A-COC | MAX | LOPU Technichian |
|---------------------------------|-----|------|-----|-------|-----|-----------------|
| Acinonyx jubatus                | 12  | 277  | 23.1| 15    | 35  | Donoghue et al., 1992 |
| Antilope cervicapra             | 10  | 73   | 7.3 | 15    | 35  | Baldassarre Unpublished |
| Cervus elaphus                  | 36  | 44   | 1.2 | N/A   | N/A | Bainbridge et al., 1999 |
| Cervus nippon nippon            | 96  | 348  | 3.6 | N/A   | N/A | Locatelli et al., 2006 |
| C. nippon pseudaxis             | 18  | 94   | 4.7 | N/A   | N/A | Locatelli et al., 2012 |
| Connochaetes taurinus           | 116 | 75   | 7.5 | N/A   | N/A | Baldassarre Unpublished |
| Cuniculus paca                  | 30  | 155  | 5.2 | N/A   | N/A | Barros et al., 2016 |
| Dama dama mhorr                 | 12  | 83   | 6.9 | 16    | 16  | Baldassarre Unpublished |
| Felis catus                     | 8   | 115  | 14.3| N/A   | N/A | Pope et al., 1993 |
| Felis silvestris ornata         | 1   | 31   | 31  | 31    | 31  | Pope et al., 1993 |
| Gazella dama mhorr              | 9   | 134  | 14.9| N/A   | N/A | Pope et al., 1993 |
| Leptailurus serval              | 9   | 234  | 26  | N/A   | N/A | Pope et al., 2005 |
| Odocoileus virginianus          | 43  | 851  | 19.8| 85    | 85  | Baldassarre Unpublished |
| Oryx gazella                    | 15  | 49   | 3.3 | 6     | 6   | Baldassarre Unpublished |
| Connochaetes taurinus           | 10  | 75   | 7.5 | 15    | 15  | Baldassarre Unpublished |
| Panthera leo                    | 3   | 26   | 8.7 | 12    | 12  | Armstrong et al., 2004 |
| Panthera onca                   | 8   | 116  | 14.5| 33    | 33  | Baldassarre/Requena Unpublished |
| Panthera tigris                 | 11  | 384  | 34.9| 62    | 62  | Crichton et al., 2002 |
| Panthera tigris                 | 16  | 456  | 28.5| 52    | 52  | Donoghue et al., 1990 |
| Priodontus viverrinus           | 2   | 107  | 53.5| N/A   | N/A | Pope et al., 1993 |
| Puma concolor                   | 12  | 421  | 35.1| 106   | 106 | Baldassarre/Requena Unpublished |
| Puma concolor                   | 7   | 140  | 20  | 52    | 52  | Miller et al., 1990 |

CONCLUSIONS

The recovery of oocytes from live animals is of great importance for the production of embryos by PIVE and also for SCNT, and LOPU is the procedure of choice for the recovery of oocytes from species and categories where transvaginal ultrasound-guided aspiration is difficult or not possible, such as small ruminants, prepubertal bovines and buffaloes, and a range of wild species. The procedure is safe and efficient, resulting in high oocyte numbers and quality. LOPU can be repeated in the same female multiple times as it doesn’t cause sequels with negative impact on the female’s reproductive life, even when performed on prepubertal or wild animals. It has realistic commercial application in sheep, goats, prepubertal cattle and buffaloes. It can also be used for species conservation.

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Fotos anexas.
Figure 3. Oócitos-Puma. 106 oocytes recovered from a single donor from Puma concolor.

Figure 4. LOPU-Búfala. First LOPU performed on 3 month old buffalo calf in Brazil, conducted by Drs. Baldassarre, Requena and Jorge Neto (Dec, 2014).

Figure 5. LOPU-Puma. Dr. Requena conducting LOPU in Puma Concolor that resulted in 106 oocytes in single collection.

Figure 6. LOPU-Saanen. Commercial LOPU conducted by Dr. Requena in goat saanen.