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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 recuperrados 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 progesterone or progestogen (eg, CIDR, Primer, sponges) which are applied with a luteolytic dose of prostaglandin or analog (e.g., 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 simultaneously (in different syringes), 36 hours before LOPU. Work performed by Baldassarre et al. (2012) did not show significant differences between both protocols, making Oneshot more attractive due to simplicity. Recently, Baldassarre et al (2012) reported an alternative protocol to the Oneshot-FSH without co-injecting eCG, by using 0.5% hyaluronic acid solution for the reconstitution of the lyophilized FSH. Hyaluronate acts as a slow release factor of FSH. The results demonstrated the efficiency of this protocol, with no differences between the control and the new treatment in the mean number of aspirated follicles (goats: 17.8 vs. 17.9; sheep 12.6 vs. 12.4) and recovered oocytes (goats: 13.7 vs. 14.0; sheep: 10.9 vs. 10.8).

In buffalo calves (2-6 months), Baldassarre et al. (2017a) tested two different superovulation protocols, both starting with the implantation of a progesterone device (Eazi-breed CIDR, Zoetis, 330 mg P4) five days before LOPU. The first, FSH-only starting 72 hours before the LOPU and consists of 6 injections with interval of 12h, totaling 140 mg of FSH. The second, with 4 injections of FSH (100mg) along with an injection of eCG (400 IU) simultaneously the last injection of FSH, 36h before the LOPU. The result of this protocol had no statistical differences in follicle number (18.0 vs. 20.3) and oocytes recovered (15.0 vs. 17.5). In bovine calves, Galli et al (2001) proposed the use of 5mg of estradiol valerate and progesterone sponge (80mg of fluorogestone) on day 0, 400 IU of eCG on days 3 and 11, 1000 IU of eCG on day 16 and LOPU in D18, showing a difference in the number of oocytes recovered from the untreated group (27.2 vs. 16, respectively). Recently, Baldassarre et al (2018) compared three treatments, all starting with the insertion of progesterone device for small ruminants (Eazi-breed CIDR, Zoetis) five days before LOPU, as well as gonadotrophin stimulation was initiated 72 h prior to the procedure. In the FSH-12 group the animals were treated intramuscularly (IM) with a total of 140 mg FSH (Folltropin-V) in 6 injections at 12 h intervals (7:00 a.m. and 7:00 p.m.). To assess the impact of more frequent stimulation of gonadotrophin, two additional protocols were tested. In the FSH-8h group, FSH was administered IM every 8 h (at 7:00 a.m., 3:00 p.m. and 11:00 p.m.) for a total dose of 180 mg (9 injections). In the FSH8h-eGG group, the animals received the same injections of FSH as FSH-8h until injection number 5, when they received 400 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 $\pm$ 16 vs. 38.5 $\pm$ 25 vs. 31.1 $\pm$ 22), mean oocytes recovered 21.6 $\pm$ 10 vs. 19.4 $\pm$ 14) and cleavage rate (66.0 $\pm$ 14 vs. 61.1 $\pm$ 11 vs. 72.2 $\pm$ 8) for FSH12h, FSH8h and FSH8h-eCG, respectively. However, FSH8h-eCG resulted in a significantly higher rate of transferable embryos (17.5 $\pm$ 8%) compared to FSH12h (8.9 $\pm$ 5%, $P < 0.05$).

In wild animals the protocols have great variations due to the particular physiology of each species. In previous studies of our 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, ICPbio 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. Locatelli et al. (2006) performed weekly LOPUs on sika deer (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 an atraumatic endoscopic

forceps. 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 laparoscopi insemination and then is then connected to the anesthesia machine ane 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 visulization. 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 passedon to the lab for finding and grading of the oocytes under the streomicroscope

LOPU in Farm Animals

LOPU presents exceptional performance in specific applications in production animals, allowing to increase the production of offspring fromfemales 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-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 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-IVEP. Used in this category, LOPU-IVEP 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-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-IVEP 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-IVEP 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 evry 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-IVEP for early reproduction of elite females before reaching puberty a sexual maturity; as well as older animals that have become unfertile with age or uncapable 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-IVEP in MOET failures, specifically animals with history of repeated failure. These are animals that have been subjected to MOET programs repeatedly and they hav always failed to produce transferable embryos for reason of a) repeated luteal regression; b) failure to superovulate (non-responders), c) failure to fertilize (only unfertile eggs are recovered). Finally, LOPU-IVEP 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 months of age, with successive collections every two weeks to seven months of age. Lohuis (1995) reports that prepubertals are ideal from the perspective of genetics companies because they reduce the intergenerational gap and thus accelerate the process of genetic improvement. They have high production of oocytes and, consequently, of embryos. This category is not eligible for MOET, with studies showing that bovine calves in this category, even when superovulated and inseminated with fresh semen by laparoscopy, have many follicles, few ovulations, and all structures recovered were non fertilized (Tervit, 1996). Baldassarre & Karatzas (2004) point out that

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.

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 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	43	772	676	15,7	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	2-6 mo.	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 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	35	Donoghue *et al*, 1992
Antidorcas marsupialis	10	73	7.3	15	Baldassarre Unpublished
Cervus elaphus	36	44	1,2	N/A	Bainbridge *et al*, 1999
Cervus nippon nippon	96	348	3,6	N/A	Locatelli *et al*, 2006
C. nippon pseudaxis	16	94	47	N/A	Locatelli *et al*, 2012
Connochaetes taurinus	118	75	7,5	N/A	Baldassarre Unpublished
Cuniculus pacas	30	155	5,2	N/A	Barros *et al*, 2016
Damaliscus pygargus phillipsi	12	83	6.9	16	Baldassarre Unpublished
Felis chaus	8	115	14,3	N/A	Pope *et al*, 1993
Felis nigripes	1	31	31	31	Pope *et al*, 1993
Felis silvestris ornata	9	134	14,9	N/A	Pope *et al*, 1993
Gazella dama mhorr	6	35	5,8	11	Berlinguer *et al*, 2008
Leptailurus serval	9	234	26	N/A	Pope *et al*, 2005
Odocoileus virginianus	43	851	19.8	85	Baldassarre Unpublished
Oryx gazela	15	49	3.3	6	Baldassarre Unpublished
Connochaetes taurinus	10	75	7.5	15	Baldassarre Unpublished
Panthera leo	3	26	8,7	12	Armstrong *et al*, 2004
Panthera onca	8	116	14,5	33	Baldassarre/Requena Unpublished
Panthera tigris	11	384	34,9	62	Crichton *et al*, 2002
Panthera tigris	16	456	28,5	52	Donoghue *et al*, 1990
Prionailurus viverrinus	2	107	53,5	N/A	Pope *et al*, 1993
Puma concolor	12	421	35,1	106	Baldassarre/Requena Unpublished
Puma concolor	7	140	20	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 bovine and buffalos, 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.

REFERENCES

- Armstrong DL, Crichton EG, Dankof SM, Schwalbach LMJ, Gardner DK, Loskutoff NM. 200 ovarian stimulation, laparoscopic oocyte retrieval, ivf and blastocyst production using sequential media in the African lion (*Panthera leo*). Reproduction, Fertility and Development. 2003;16(2): 221-221.
- Armstrong DT, Holm P, Irvine B, Petersen BA, Stubbings RB, McLean D, Seamark RF. Pregnancies and live birth from in vitro fertilization of calf oocytes collected by laparoscopic follicular aspiration. Theriogenology. 1992; 38(4); 667-678.
- Bainbridge DRJ, Catt SL, Evans G, Jabbour HN. Successful in vitro fertilization of in vivo matured oocytes aspirated laparoscopically from red deer hinds (*Cervus elaphus*). Theriogenology. 1999; 51(5): 891-898.
- Baldassarre H, De Matos DG, Furnus CC, Castro TE, Fischer EC. Technique for efficient recovery of sheep oocytes by laparoscopic folliculocentesis. Animal Reproduction Science. 1994; 35(1-2):145-150.
- Baldassarre H, Furnus CC, De Matos DG, Pessi H. In vitro production of sheep embryos using laparoscopic folliculocentesis: alternative gonadotrophin treatments for stimulation of oocyte donors. Theriogenology. 1996; 45(3): 707-717.
- Baldassarre H, Rao KM, Neveu N, Brochu E, Begin I, Behboodi E, Hockley DK. Laparoscopic ovum pick-up followed by in vitro embryo production for the reproductive rescue of aged goats of high genetic value. Reproduction, Fertility and Development. 2007; 19(5): 612-616.
- Baldassarre H, Karatzas CN. Advanced assisted reproduction technologies (ART) in goats. Animal Reproduction Science- 2004; 82: 255-266.
- Baldassarre H. Practical aspects for implementing in vitro embryo production and cloning programs in sheep and goats. Anim. Reprod. 2012; 9: 188-194.
- Baldassarre H, Currin L, Michalovic L, Glanzner W, Gutierrez K, Bohrer R, Bordignon V. 159 in vitro embryo production from holstein calf oocytes recovered by laparoscopic ovum pickup. Reproduction, Fertility and Development. 2016; 28(2): 209-209.
- Baldassarre H, Littkemann M, Currin L, Macedo MP, Gutierrez K, Bordignon V. Efficient Shortening of Generation Interval by Applying Laparoscopic Ovum Pick-up and in vitro Embryo Production Technologies in Mediterranean Buffalo of 2-6 Months of Age. IX Symporsium of the International Buffalo Federation. Campeche, Mexico - Nov. 20-25. 2017a.
- Baldassare H, Requena LA, Carelli JB, Salomão Júnior JA, Rodrigues MG, Ferreira SAP, Traldi AS, Jorge Neto PN. Laparoscopic ovum pick-up is a safe procedure for the collection of oocytes for preservation efforts in Pumas (*Puma concolor*). Animal Reproduction. 2017b; 14: 780.

- Baldassarre H, Currin L, Michalovic L, Bellefleur AM, Gutierrez K, Mondadori RG, Lopez R. Interval of gonadotropin administration for in vitro embryo production from oocytes collected from Holstein calves between 2 and 6 months of age by repeated laparoscopy. Theriogenology. 2018; 116: 64-70.
- Barros FFC, Teixeira PPM, Uscategui RAR, Coutinho LN, Brito MBS, Kawanami AE, Vicente WRR. Laparoscopic ovum pick-up in spotted paca (Cuniculus pacas). Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2016; 68(4): 858-864.
- Berlinguer F, González R, Succu S, Del Olmo A, Garde JJ, Espeso G, Roldan ER. In vitro oocyte maturation, fertilization and culture after ovum pick-up in an endangered gazelle (*Gazella dama mhorr*). Theriogenology. 2008; 69(3):349-359.
- Campos FGCM, Roll S. Complicações do acesso abdominal e do pneumoperitônio em cirurgia laparoscópica: causas, prevenção e tratamento. Rev Bras Videocir. 2003; 1: 21-8.
- Crichton EG, Bedows E, Miller-Lindholm AK, Baldwin DM, Armstrong DL, Graham LH, Vajta, G. Efficacy of porcine gonadotropins for repeated stimulation of ovarian activity for oocyte retrieval and in vitro embryo production and cryopreservation in Siberian tigers (Panthera tigris altaica). Biology of reproduction. 2003; 68(1):105-113.
- Donoghue AM, Howard JG, Byers AP, Goodrowe KL, Bush M, Blumer E, Wildt DE. Correlation of sperm viability with gamete interaction and fertilization in vitro in the cheetah (Acinonyx jubatus). Biology of Reproduction. 1992; 46(6):1047-1056.
- Galli C, Crotti G, Notari C, Turini P, Duchi R, Lazzari G. Embryo production by ovum pick up from live donors. Theriogenology. 2001; *55*(6): 1341-1357.
- Koeman J, Keefer CL, Baldassarre H, Downey BR. Developmental competence of prepubertal and adult goat oocytes cultured in semi-defined media following laparoscopic recovery. Theriogenology. 2003; 60(5): 879-889.
- Locatelli Y, Vallet JC, Huyghe FP, Cognie Y, Legendre X, Mermillod P. Laparoscopic ovum pick-up and in vitro production of sika deer embryos: effect of season and culture conditions. Theriogenology. 2006; 66(5): 1334-1342.
- Locatelli Y, Hendriks A, Vallet JC, Baril G, Duffard N, Bon N, Legendre X. Assessment LOPU-IVF in Japanese sika deer (*Cervus nippon nippon*) and application to Vietnamese sika deer (*Cervus nippon pseudaxis*) a related subspecies threatened with extinction. Theriogenology. 2012; 78(9): 2039-2049.
- Lohuis MM. Potential benefits of bovine embryo-manipulation technologies to genetic improvement programs. Theriogenology. 1995; 43(1): 51-60.
- Mendes CS, Barbosa LP, de Araújo ML, Pinheiro EEG, Lents MP, da Silva Amorim L, Santana ALA. Follicular response and oocyte production following variations in ovarian stimulation in goats. Theriogenology. 2018; 108: 88-96.
- Miller AM, Roelke ME, Goodrowe KL, Howard J, Wildt DE. Oocyte recovery, maturation and fertilization in vitro in the puma (*Felis concolor*). Journal of reproduction and fertility. 1990; 88(1): 249-258.
- Pope CE, Keller GL, Dresser BL. In vitro fertilization in domestic and non-domestic cats including sequences of early nuclear events, development in vitro, cryopreservation and successful intra-and interspecies embryo transfer. Journal of reproduction and fertility. Supplement. 1993; 47: 189-201.
- Pope CE, Gómez MC, Cole A, Dumas C, Dresser BL. 229 oocyte recovery, in vitro fertilization and embryo transfer in the serval (Leptailurus serval). Reproduction, Fertility and Development. 2005; 18(2): 223-223.
- Taneja M, Bols PE, de Velde AV, Ju JC, Schreiber D, Tripp MW, Yang X. Developmental competence of juvenile calf oocytes in vitro and in vivo: influence of donor animal variation and repeated gonadotropin stimulation. Biology of reproduction, 2000; 62(1), 206-213.
- Tervit HR. Laparoscopy/laparotomy oocyte recovery and juvenile breeding. Animal Reproduction Science. 1996; 42(1-4): 227-238.

Fotos anexas.

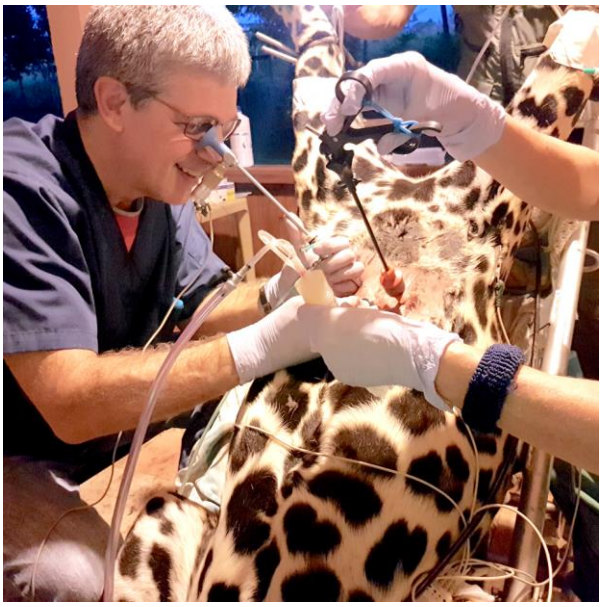

Figure 1. LOPU-Jaguar. Dr. Baldassarre conducting LOPU in jaguars (Panthera onca)

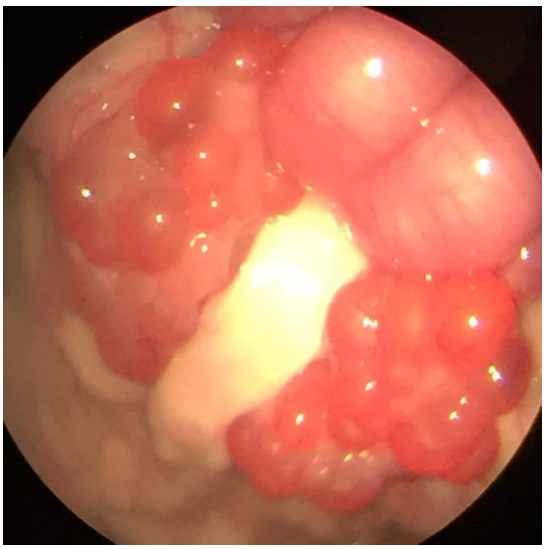

Figure 2. Ovario-Puma. Superestimated Ovary of Puma concolor, that result in 106 oocytes in a single collection.

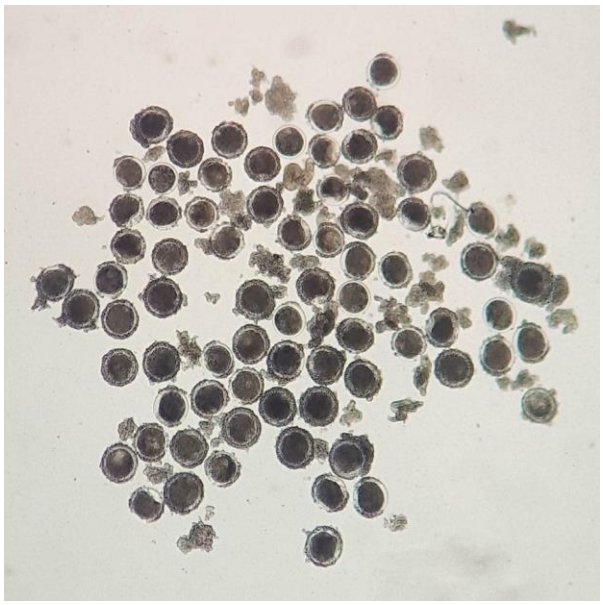

Figure 3. Oócitos-Puma.106 oocytes recovered from a single donor from Puma concolor.

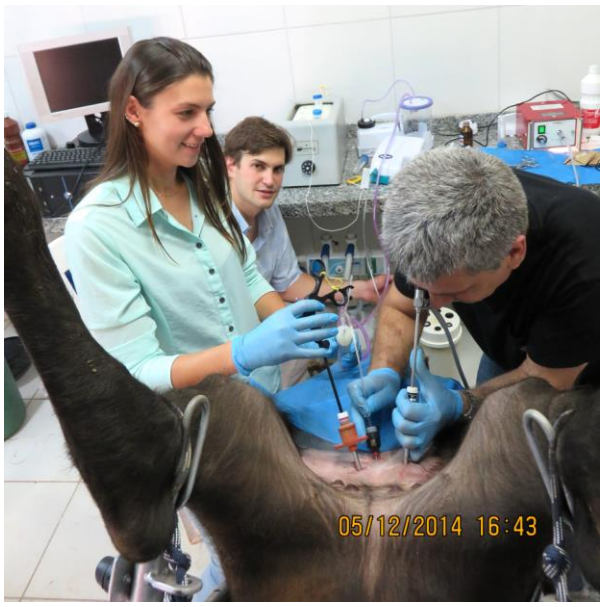

Figure 4. LOPU-Búfala. First LOPU performed on 3 month old buffalo calf in Brazil , conducted by Drs. Baldassarre, Requena and Jorge Neto (Dec, 2014).

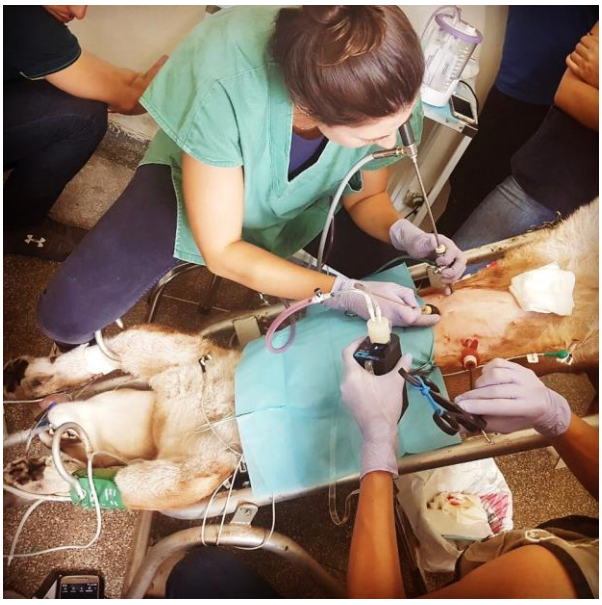

Figure 5. LOPU-Puma. Dr. Requena conducting LOPU in Puma Concolor that resulted in 106 oocytes in single collection

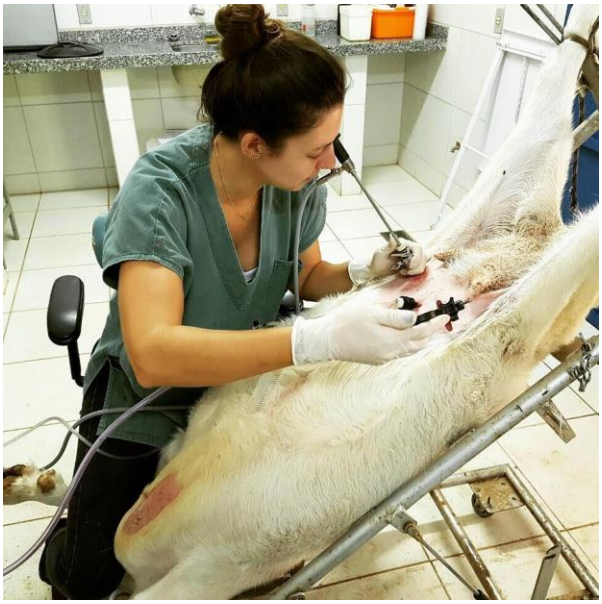

Figure 6. LOPU-Saanen.Commercial LOPU conducted by Dr. Requena in goat saanen.