



**JORNADAS**  
**ALTBiotech** RepGen

Estação Zootécnica Nacional  
Vale de Santarém

**RECURSOS GENÉTICOS ANIMAIS**  
**E BIOTECNOLOGIAS:** 13 e 14 de dezembro  
**PROJEÇÃO PARA O FUTURO** 2019

OPU  
10.00 - 3.40  
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*“Ovum Pick-Up (OPU)  
Alternativa emergente  
para a produção  
de embriões”*



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### What's OPU? *Ovum Pick-Up*

Ultrasound-guided puncture and aspiration of ovarian follicles





## OPU (Ovum Pick-Up)

- Cyclic, non-cyclic and pregnant animals (1<sup>st</sup> third)
- No response to hormonal stimuli
- With no genetic reproductive disorders
- Heifers and prepubertal heifers (from 6-8 months)
- OPU/IVEP a viable alternative to recovery animal genetic resources



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### OVUM PICK-UP (OPU) & IN VITRO EMBRYO PRODUCTION (IVEP)

Bovine *In Vitro* Embryo Production in Europe in 2017 (OPU/IVEP)

| Country            | Sessions     | Oocytes       | Oocytes/<br>Session | Embryos      | Embryos/<br>Session | Stimulated<br>sessions (%) | Sexed semen<br>(%) | Dairy breeds<br>(%) |
|--------------------|--------------|---------------|---------------------|--------------|---------------------|----------------------------|--------------------|---------------------|
| Finland            | 455          | 3748          | 8,24                | 1211         | 2,66                | 77,4 %                     | 17,8 %             | 100,0 %             |
| France             | 691          | 6171          | 8,93                | 1756         | 2,54                | 99,0 %                     | 18,4 %             | 95,7 %              |
| Germany            | 1020         | 13880         | 13,61               | 1794         | 1,76                | 55,2 %                     | 0,0 %              | 98,3 %              |
| Italy              | 482          | 4819          | 10,00               | 818          | 1,70                | 21,6 %                     | 40,2 %             | 100,0 %             |
| Netherlands        | 7345         | 83421         | 11,36               | 16695        | 2,27                | 80,0 %                     | 0,0 %              | 100,0 %             |
| Poland             | 14           | 89            | 6,36                | 34           | 2,43                | 0,0 %                      | 78,6 %             | 100,0 %             |
| Russian Federation | 7758         | 87120         | 11,23               | 26762        | 3,45                | 0,0 %                      | 73,6 %             | 42,2 %              |
| Spain              | 839          | 11122         | 13,26               | 2746         | 3,27                | 12,0 %                     | 65,8 %             | 86,1 %              |
| Switzerland        | 41           | 312           | 7,61                | 81           | 1,98                | 0,0 %                      | 56,1 %             | 100,0 %             |
| United Kingdom     | 39           | 415           | 10,64               | 74           | 1,90                | 0,0 %                      | 7,7 %              | 7,7 %               |
| <b>Total</b>       | <b>18684</b> | <b>211097</b> | <b>11,30</b>        | <b>51971</b> | <b>2,78</b>         | <b>49,0 %</b>              | <b>35,8 %</b>      | <b>74,9 %</b>       |

**11.30** oocytes/session      1. Russia: 41.5% OPU, 41.3% oocytes, 51.5% embryos  
**2.78** embryos/session      2. Netherlands: 39.3%, 39.5%, 32.1%

Mikkola M. Commercial Embryo Transfer Activity in Europe 2017  
 34<sup>th</sup> Annual Meeting AETE. Nantes, France. 2018

**Bovine *In Vitro* Embryo Production in Europe in 2018 (OPU/IVEP)**

| Country            | Dairy          |                 |               |              |                |                 |                |               | Beef           |                 |              |              |                |                 |            |            | All           |                |               |     |
|--------------------|----------------|-----------------|---------------|--------------|----------------|-----------------|----------------|---------------|----------------|-----------------|--------------|--------------|----------------|-----------------|------------|------------|---------------|----------------|---------------|-----|
|                    | Non-stimulated |                 |               |              | Stimulated     |                 |                |               | Non-stimulated |                 |              |              | Stimulated     |                 |            |            | Sessions      | Oocytes        | Embryos       |     |
|                    | OPU Conv semen | OPU Sexed semen | Oocytes       | Embryos      | OPU Conv semen | OPU Sexed semen | Oocytes        | Embryos       | OPU Conv semen | OPU Sexed semen | Oocytes      | Embryos      | OPU Conv semen | OPU Sexed semen | Oocytes    | Embryos    |               |                |               |     |
| Estonia            | 0              | 0               | 0             | 0            | 0              | 0               | 0              | 0             | 0              | 0               | 0            | 0            | 0              | 0               | 8          | 74         | 24            | 8              | 74            | 24  |
| Finland            | 368            | 0               | 1 809         | 67           | 315            | 0               | 2 946          | 385           | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 683            | 4 755         | 452 |
| France             | 206            | 13              | 924           | 214          | 277            | 80              | 3 928          | 831           | 0              | 0               | 0            | 0            | 46             | 0               | 456        | 166        | 622           | 5 308          | 1 211         |     |
| Germany            | 809            | 0               | 11 844        | 2 621        | 0              | 0               | 0              | 0             | 20             | 0               | 337          | 89           | 0              | 0               | 0          | 0          | 0             | 829            | 12 181        |     |
| Italy              | 119            | 0               | 1 327         | 188          | 0              | 0               | 0              | 0             | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 119            | 1 327         |     |
| Netherlands        | 694            | 85              | 11 300        | 2 502        | 9 750          | 0               | 104 103        | 27 300        | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 10 529         | 115 403       |     |
| Poland             | 4              | 10              | 112           | 62           | 0              | 33              | 324            | 128           | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 47             | 436           |     |
| Romania            | 0              | 0               | 0             | 0            | 4              | 0               | 5              | 1             | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 4              | 5             |     |
| Russian Federation | 0              | 831             | 4 621         | 936          | 0              | 0               | 0              | 0             | 0              | 300             | 2 461        | 723          | 0              | 0               | 0          | 0          | 0             | 1 131          | 7 082         |     |
| Serbia             | 11             | 7               | 76            | 28           | 8              | 15              | 128            | 32            | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 41             | 204           |     |
| Spain              | 0              | 0               | 0             | 0            | 0              | 50              | 770            | 151           | 68             | 0               | 473          | 239          | 0              | 0               | 0          | 0          | 0             | 118            | 1 243         |     |
| Switzerland        | 40             | 29              | 841           | 145          | 0              | 0               | 0              | 0             | 0              | 0               | 0            | 0            | 0              | 0               | 0          | 0          | 0             | 69             | 841           |     |
| <b>Grand Total</b> | <b>2 251</b>   | <b>975</b>      | <b>32 854</b> | <b>6 763</b> | <b>10 354</b>  | <b>178</b>      | <b>112 204</b> | <b>28 828</b> | <b>88</b>      | <b>300</b>      | <b>3 271</b> | <b>1 051</b> | <b>46</b>      | <b>8</b>        | <b>530</b> | <b>190</b> | <b>14 200</b> | <b>148 859</b> | <b>36 832</b> |     |

**10.48 oocytes/session**      1. Netherlands: 74% OPUs, 77.5% oocytes, 81% embryos  
**2.59 embryos/session**      2. Russia: 8%, 4.75%, 4.62%

Total OPU:      2017 (18,684) vs. 2018 (14,200): 24.8% ↓  
 Total oocytes:    2017 (211,097) vs. 2018 (148,859): 29.5% ↓  
 Total embryos:    2017 (51,971) vs. 2018 (36,832): 29.1% ↓

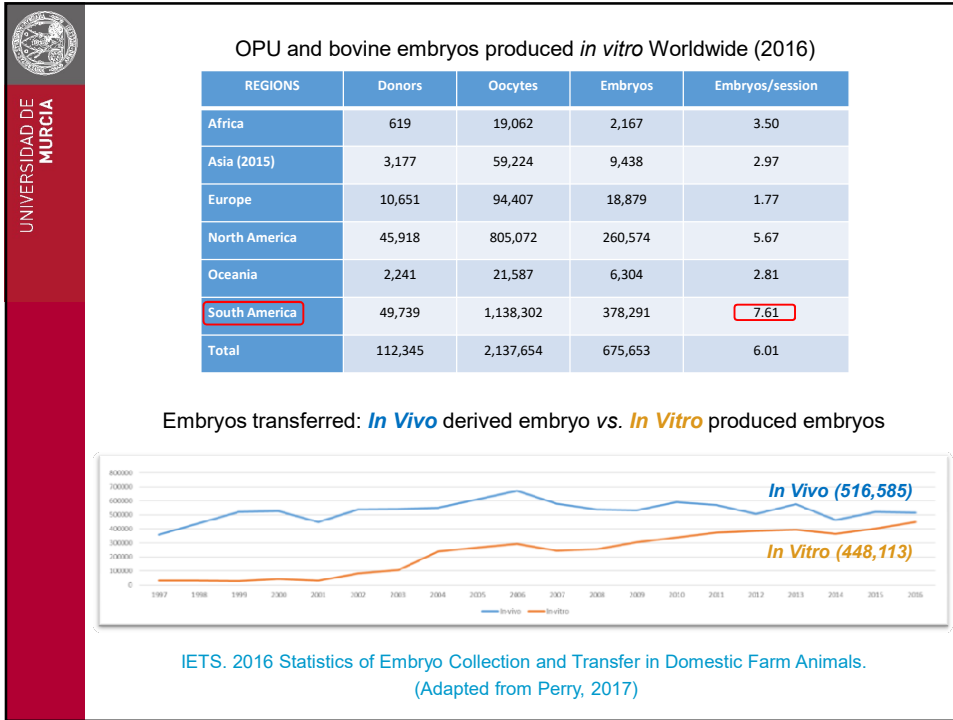
Mikkola M. Commercial Embryo Transfer Activity in Europe 2018.  
 35<sup>th</sup> Annual Meeting AETE. Murcia, Spain. 2019


**Bovine *In Vitro* Embryo Transfer in Europe in 2018**

| Country            | OPU           |               |                |             | Abattoir  |            | Total transfers IVP |
|--------------------|---------------|---------------|----------------|-------------|-----------|------------|---------------------|
|                    | Fresh         | Frozen        | Frozen foreign | OPU Exports | Fresh     | Frozen     |                     |
| Belgium            | 0             | 0             | 1 315          | 0           | 0         | 0          | 1 315               |
| Estonia            | 17            | 0             | 0              | 0           | 0         | 0          | 17                  |
| Finland            | 19            | 457           | 0              | 0           | 0         | 0          | 476                 |
| France             | 369           | 348           | 54             | 88          | 0         | 0          | 859                 |
| Germany            | 2 007         | 909           | 0              | 0           | 0         | 0          | 2 916               |
| Hungary            | 0             | 0             | 105            | 0           | 0         | 0          | 105                 |
| Italy              | 126           | 15            | 0              | 0           | 0         | 0          | 141                 |
| Netherlands        | 11 124        | 10 354        | 0              | 0           | 0         | 0          | 21 478              |
| Poland             | 23            | 17            | 0              | 0           | 0         | 0          | 40                  |
| Romania            | 0             | 1             | 0              | 0           | 12        | 3          | 16                  |
| Russian Federation | 111           | 521           | 0              | 0           | 0         | 0          | 632                 |
| Serbia             | 12            | 37            | 0              | 0           | 0         | 0          | 49                  |
| Spain              | 59            | 158           | 0              | 0           | 49        | 282        | 548                 |
| Switzerland        | 0             | 14            | 81             | 0           | 0         | 0          | 95                  |
| United Kingdom     | 0             | 117           | 0              | 0           | 0         | 0          | 117                 |
| <b>Grand Total</b> | <b>13 867</b> | <b>12 948</b> | <b>1 555</b>   | <b>88</b>   | <b>61</b> | <b>285</b> | <b>28 804</b>       |

Netherlands: 74.6% ETs

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 35<sup>th</sup> Annual Meeting AETE. Murcia, Spain. 2019





### Bovine *In Vitro* Embryo Production (OPU/IVPE)

| REGION / COUNTRY     | BOVINE OVUM PICK-UP EMBRYOS |       |            |       |                |       |                  |      |                |       |            |       |                |        |            |       |                |        |                      |        |        |       |      |       |
|----------------------|-----------------------------|-------|------------|-------|----------------|-------|------------------|------|----------------|-------|------------|-------|----------------|--------|------------|-------|----------------|--------|----------------------|--------|--------|-------|------|-------|
|                      | CONVENTIONAL SEMEN          |       |            |       |                |       | SEX SORTED SEMEN |      |                |       |            |       | OOCYTES        |        |            |       |                |        | TRANSFERABLE EMBRYOS |        |        |       |      |       |
|                      | NON-STIMULATED              |       | STIMULATED |       | NON-STIMULATED |       | STIMULATED       |      | NON-STIMULATED |       | STIMULATED |       | NON-STIMULATED |        | STIMULATED |       | NON-STIMULATED |        | STIMULATED           |        |        |       |      |       |
|                      | DAIRY                       | BEEF  | TOTAL      | DAIRY | BEEF           | TOTAL | DAIRY            | BEEF | TOTAL          | DAIRY | BEEF       | TOTAL | DAIRY          | BEEF   | TOTAL      | DAIRY | BEEF           | TOTAL  | DAIRY                | BEEF   | TOTAL  | DAIRY | BEEF | TOTAL |
| <b>Europe</b>        | 0                           | 0     | 0          | 1     | 0              | 1     | 0                | 0    | 0              | 0     | 0          | 0     | 0              | 0      | 0          | 7     | 0              | 7      | 0                    | 0      | 0      | 1     | 0    | 1     |
| Austria              | 0                           | 0     | 0          | 1     | 0              | 1     | 0                | 0    | 0              | 0     | 0          | 0     | 687            | 0      | 687        | 790   | 0              | 790    | 342                  | 0      | 342    | 320   | 0    | 320   |
| Finland              | 0                           | 0     | 0          | 524   | 29             | 553   | 0                | 0    | 0              | 58    | 0          | 58    | 0              | 0      | 0          | 4833  | 538            | 5331   | 0                    | 0      | 0      | 1370  | 258  | 1628  |
| France               | 1311                        | 41    | 1352       | 0     | 0              | 0     | 0                | 0    | 0              | 0     | 0          | 0     | 11496          | 1009   | 12505      | 0     | 0              | 0      | 1843                 | 76     | 1919   | 0     | 0    | 0     |
| Germany              | 294                         | 219   | 513        | 0     | 0              | 0     | 68               | 0    | 68             | 0     | 0          | 0     | 4202           | 2995   | 7197       | 0     | 0              | 0      | 495                  | 1015   | 1510   | 0     | 0    | 0     |
| Italy                | 0                           | 0     | 0          | 4726  | 0              | 4726  | 0                | 0    | 0              | 0     | 0          | 0     | 0              | 0      | 0          | 45662 | 0              | 45662  | 0                    | 0      | 0      | 8385  | 0    | 8385  |
| Netherlands          | 9                           | 0     | 9          | 4     | 0              | 4     | 0                | 0    | 0              | 0     | 0          | 0     | 47             | 0      | 47         | 17    | 0              | 17     | 24                   | 0      | 24     | 10    | 0    | 10    |
| Poland               | 0                           | 0     | 0          | 0     | 0              | 0     | 3529             | 0    | 3529           | 0     | 0          | 0     | 6076           | 0      | 6076       | 0     | 0              | 0      | 911                  | 0      | 911    | 0     | 0    | 0     |
| Russian Federation   | 6                           | 0     | 6          | 8     | 0              | 8     | 0                | 0    | 0              | 0     | 0          | 0     | 24             | 0      | 24         | 56    | 0              | 56     | 21                   | 0      | 21     | 24    | 0    | 24    |
| Serbia               | 137                         | 102   | 239        | 25    | 31             | 56    | 329              | 5    | 334            | 86    | 19         | 105   | 6569           | 1052   | 7621       | 1816  | 455            | 2271   | 1472                 | 381    | 1853   | 543   | 183  | 726   |
| Spain                | 53                          | 277   | 330        | 238   | 217            | 455   | 15               | 1    | 16             | 85    | 0          | 85    | 697            | 2298   | 2995       | 1836  | 1295           | 3131   | 127                  | 583    | 710    | 457   | 248  | 705   |
| United Kingdom       | 53                          | 277   | 330        | 238   | 217            | 455   | 15               | 1    | 16             | 85    | 0          | 85    | 697            | 2298   | 2995       | 1836  | 1295           | 3131   | 127                  | 583    | 710    | 457   | 248  | 705   |
| Europe Total         | 1913                        | 639   | 2552       | 5637  | 277            | 5914  | 1931             | 6    | 1937           | 229   | 19         | 248   | 29798          | 7354   | 37152      | 54987 | 2288           | 57255  | 5025                 | 2055   | 7080   | 11110 | 689  | 11799 |
| <b>North America</b> | 0                           | 0     | 0          | 2629  | 171            | 2800  | 0                | 0    | 0              | 994   | 41         | 1035  | 0              | 0      | 0          | 35362 | 2309           | 37671  | 0                    | 0      | 0      | 14887 | 1158 | 16045 |
| Austria              | 0                           | 0     | 0          | 2629  | 171            | 2800  | 0                | 0    | 0              | 994   | 41         | 1035  | 0              | 0      | 0          | 35362 | 2309           | 37671  | 0                    | 0      | 0      | 14887 | 1158 | 16045 |
| Canada               | 0                           | 1170  | 1170       | 0     | 0              | 0     | 0                | 5    | 5              | 0     | 0          | 0     | 0              | 0      | 0          | 12450 | 12450          | 0      | 0                    | 0      | 0      | 5700  | 5700 |       |
| Mexico               | 22823                       | 18085 | 40908      | 0     | 0              | 0     | 0                | 0    | 0              | 0     | 0          | 0     | 392273         | 362688 | 754961     | 0     | 0              | 0      | 121317               | 117512 | 238829 | 0     | 0    | 0     |
| United States        | 22823                       | 18085 | 40908      | 0     | 0              | 0     | 0                | 0    | 0              | 0     | 0          | 0     | 392273         | 362688 | 754961     | 0     | 0              | 0      | 121317               | 117512 | 238829 | 0     | 0    | 0     |
| North America Total  | 22823                       | 19255 | 42078      | 2629  | 171            | 2800  | 0                | 5    | 5              | 994   | 41         | 1035  | 392273         | 375138 | 767411     | 35362 | 2309           | 37661  | 121317               | 123212 | 244529 | 14887 | 1158 | 16045 |
| <b>South America</b> | 8                           | 3042  | 3050       | 0     | 0              | 0     | 12               | 27   | 39             | 0     | 0          | 0     | 925            | 67071  | 67996      | 0     | 0              | 0      | 341                  | 19893  | 20234  | 0     | 0    | 0     |
| Argentina            | 8                           | 3042  | 3050       | 0     | 0              | 0     | 12               | 27   | 39             | 0     | 0          | 0     | 925            | 67071  | 67996      | 0     | 0              | 0      | 341                  | 19893  | 20234  | 0     | 0    | 0     |
| Brazil               | 0                           | 289   | 289        | 0     | 0              | 0     | 468              | 0    | 468            | 0     | 0          | 0     | 6966           | 7355   | 14321      | 0     | 0              | 0      | 2352                 | 2215   | 4567   | 0     | 0    | 0     |
| Dominican Republic   | 0                           | 371   | 371        | 0     | 0              | 0     | 470              | 0    | 470            | 0     | 0          | 0     | 7049           | 6612   | 13661      | 0     | 0              | 0      | 1581                 | 1586   | 3167   | 0     | 0    | 0     |
| Panama               | 0                           | 371   | 371        | 0     | 0              | 0     | 470              | 0    | 470            | 0     | 0          | 0     | 7049           | 6612   | 13661      | 0     | 0              | 0      | 1581                 | 1586   | 3167   | 0     | 0    | 0     |
| Peru                 | 4711                        | 319   | 5030       | 0     | 0              | 0     | 1121             | 17   | 1138           | 0     | 0          | 0     | 46703          | 2735   | 49438      | 0     | 0              | 0      | 2852                 | 247    | 3099   | 0     | 0    | 0     |
| South America Total  | 25677                       | 21947 | 47624      | 0     | 0              | 0     | 2071             | 44   | 2115           | 0     | 0          | 0     | 507443         | 540859 | 1138302    | 0     | 0              | 0      | 193963               | 184328 | 378291 | 0     | 0    | 0     |
| <b>Grand Total</b>   | 51519                       | 41159 | 92678      | 8266  | 726            | 8992  | 4025             | 377  | 4402           | 1223  | 109        | 1332  | 1026776        | 917618 | 1944394    | 96389 | 21192          | 117581 | 322246               | 362337 | 627482 | 25997 | 3452 | 29449 |

**Brazil:** 25.47 oocytes/session (2016) 8.91 embryos/session (6.3 emb ↑)

**Europe:** 10.48 oocytes/session (2018) 2.59 embryos/session

**1. Brazil:** 42.1% OPUs, 51% oocytes, 55.3% embryos

**2. USA:** 44.3%, 38.8%, 38.1%

IETS. 2016 Statistics of Embryo Collection and Transfer in Domestic Farm Animals. 2017

| REGION / COUNTRY   | BOVINE OPU IVP EMBRYO TRANSFER |          |         |        |          |
|--------------------|--------------------------------|----------|---------|--------|----------|
|                    | FRESH                          | FROZEN   |         | TOTAL  | EXPORTED |
|                    |                                | DOMESTIC | FOREIGN |        |          |
| Africa             | 379                            | 77       | 169     | 625    | 0        |
| South Africa       | 379                            | 77       | 169     | 625    | 0        |
| Asia               | 0                              | 0        | 0       | 0      | 0        |
| Europe             | 10424                          | 3343     | 292     | 14059  | 0        |
| Finland            | 61                             | 226      | 0       | 287    | 0        |
| France             | 526                            | 543      | 2       | 1071   | 0        |
| Germany            | 1556                           | 265      | 0       | 1821   | 0        |
| Italy              | 479                            | 372      | 0       | 851    | 0        |
| Luxembourg         | 0                              | 0        | 50      | 50     | 0        |
| Netherlands        | 6448                           | 1242     | 0       | 7690   | 0        |
| Poland             | 17                             | 10       | 0       | 27     | 0        |
| Russian Federation | 81                             | 47       | 0       | 128    | 0        |
| Serbia             | 5                              | 30       | 0       | 35     | 0        |
| Spain              | 1196                           | 413      | 149     | 1758   | 0        |
| Switzerland        | 0                              | 0        | 91      | 91     | 0        |
| United Kingdom     | 55                             | 195      | 0       | 250    | 0        |
| North America      | 80825                          | 50672    | 0       | 131497 | 5165     |
| Canada             | 6375                           | 1824     | 0       | 8199   | 494      |
| Mexico             | 5250                           | 315      | 0       | 5565   | 0        |
| United States      | 69200                          | 48533    | 0       | 117733 | 4671     |
| Oceania            | 1334                           | 1516     | 0       | 2850   | 0        |
| Australia          | 3398                           | 263      | 0       | 3661   | 0        |
| New Zealand        | 1334                           | 1439     | 0       | 2773   | 0        |
| South America      | 230263                         | 65235    | 0       | 295498 | 0        |
| Argentina          | 9531                           | 3120     | 0       | 12651  | 0        |
| Brazil             | 215195                         | 60723    | 0       | 275918 | 0        |
| Dominican Republic | 1241                           | 0        | 0       | 1241   | 0        |
| Panama             | 2222                           | 367      | 0       | 2589   | 0        |
| Peru               | 2074                           | 1025     | 0       | 3099   | 0        |
| Grand Total        | 326623                         | 121029   | 461     | 448113 | 5165     |


Brazil: 61.6% ETS

IETS. 2016 Statistics of Embryo Collection and Transfer in Domestic Farm Animals. 2017

## Factors affecting OPU results

**Technical factors**

- **Follicular Aspiration Needles**
  - Disposables
  - 18-20 G
  - 40-75 cm long
- **Vacuum Pump**
  - Depends aspiration device, length and diameter tubing, size and type collection vessel & needle diameter)
  - > 50 and < 120 mm Hg
  - Flow rate: 15-25 ml/min
- **Ultrasonography**
  - Convex or Micro-convex transducers (6-8 Mhz)
  - Linear probes, lower OPU efficiency (10-20%)
  - Aloka-500, Hitachi-EUB, Capasee-Toshiba, Medison Sonovet-600, Pie-Medical Falco-Vet, Exago-ECM, Easi-Scan BCF, GE, ...
  - Handgrips
- **OPU Team and Operator Experience**
  - One or two person. Same people
  - Experience (operator and assistant) significant effect in number and quality of collected oocytes

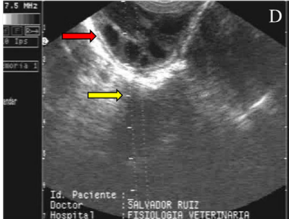


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## Factors affecting OPU results

### Biological factors (I)

- **Frequency and timing of follicle puncture**
  - OPU 2/week (Mon & Thurs, 3-4 days interval)
  - OPU 1/week
- **Physiological status and body condition of the donor**
  - Selected healthy heifers with genetic potential for production traits
  - OPU in non-productive periods. Undernutrition has a negative effect
  - OPU exceptions: pregnant animals (after 3<sup>rd</sup>-4<sup>th</sup> mo), ovarian hypoplasia or immediate post-partum
- **Individual variations**
  - Donor animal is a major source of variation both the management and genetics
  - In human medicine, plasma Anti-Müllerian Hormone (AMH) levels is an important predictive parameter for assistant reproductive technologies (ARTs) success
  - Currently, controversy between authors about AMH in bovine. However, AMH can help to identify groups of very good or very poor oocyte donors
  - More studies must be conducted to determine the role of AMH levels in OPU/IVEP procedures in cattle
- **Donor age**
  - Very young and very old donor ages are reported as problematic situations
  - Prepubertal and aging donors commonly have poor-quality oocytes
  - Virtually all female cattle starting from 2 mo of age can be oocyte donors by follicular aspiration



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## OPU/IVEP

(Chaubal *et al.*, 2006)


| Treatment                      | Follicles aspirated | Oocytes retrieved | Oocytes cultured | Blastocysts D7 | Blastocysts D7 + 8 |
|--------------------------------|---------------------|-------------------|------------------|----------------|--------------------|
| Per cow per session (rows 1-5) |                     |                   |                  |                |                    |
| OPU 1/w                        | 7.8 ± 2.4 aH        | 4.6 ± 1.9 aH      | 4.5 ± 1.9 aH     | 0.6 ± 0.8 aH   | 0.9 ± 0.9 aH       |
| OPU 2/w                        | 6.5 ± 2.4 a         | 3.9 ± 2.1 a       | 3.8 ± 2.0 a      | 0.7 ± 0.7 a    | 0.8 ± 0.9 a        |
| DFR-OPU 1/w                    | 7.3 ± 2.4 aH        | 5.3 ± 2.2 aH      | 5.2 ± 2.1 aH     | 1.2 ± 1.3 bI   | 1.4 ± 1.6 bcHI     |
| DFR-FSH-OPU 1/w                | 16.0 ± 5.0 bI       | 10.6 ± 4.5 bI     | 10.1 ± 4.4 bI    | 2.1 ± 1.2 bI   | 2.4 ± 1.4 bI       |
| FSH-OPU 2/w                    | 7.6 ± 2.9 a         | 4.9 ± 2.6 a       | 4.8 ± 2.6 a      | 0.9 ± 1.1 a    | 1.1 ± 1.4 ac       |

(Viana *et al.*, 2004)

Performance under in vitro culture<sup>a</sup> (n and %) of oocytes recovered in vivo by follicle aspiration performed once (TVFA-1x) or twice (TVFA-2x) weekly, or from ovaries recovered from slaughterhouse (control) (Gyr, *B. Indicus*)

|                         | TVFA-1x |        | TVFA-2x |        | Control |        |
|-------------------------|---------|--------|---------|--------|---------|--------|
|                         | n/total | %      | n/total | %      | n/total | %      |
| Selected for IVF        | 418/564 | 74.1 a | 541/657 | 82.3 b | 663     | –      |
| Cleaved                 | 238/348 | 68.4 a | 339/494 | 68.6 a | 392/663 | 59.1 a |
| Greater than four cells | 139/319 | 43.6 a | 204/424 | 48.1 a | –       | –      |
| Blastocysts             | 69/319  | 21.6 a | 135/424 | 31.8 b | 139/663 | 21.0 a |

Columns with different letters differ significantly ( $P < 0.05$ ).  
<sup>a</sup> Replicates presenting problems during in vitro culture not related to the oocytes were excluded.

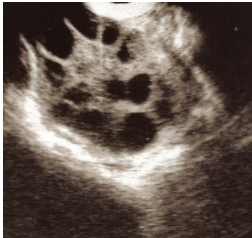



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## Factors affecting OPU results

### Biological factors (II)

- **Climate and season**
  - Heat stress suppress follicular dominance, causing follicle growth-related changes
  - Number of follicles 3-8 mm in diameter per ovary and number oocytes after aspiration of follicles is higher in winter compared with summer
  - *B. indicus* cattle have shown better reproductive performance than *B. taurus* in tropical and subtropical regions
- **Hormonal stimulation**
  - Gonadotropin stimulation ranging from a full superovulatory dose to shorter treatments (2-3 days, 1-2 injec./day) in presence of a P4 releasing device or a CL
  - Gonadotropins increasing size of small follicles and to the acquisition of a higher developmental competence of the oocytes
  - In prepubertal calves gonadotropin stimulation is required to obtain an acceptable level of developmental competence
  - Use of gonadotropin stimulation does not seem to be effective in *B. indicus* donors; it is not used in large scale programs






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## Factors affecting OPU results

### Biological factors (III)

- **Donor breed**
  - In *Bos taurus* not significant variation among breeds
  - Significant differences between *B. indicus* breeds and *indicus-taurus* donors
  - *B. indicus* breeds tend to have, on average, more follicular waves, as well as a greater number of follicles >5 mm per wave compared to *B. taurus* breeds
  - Nelore cows have smaller dominant follicles and CL, and shorter estrus than *B. taurus* breeds. Physiological basis for the number of follicles in Nelore cattle has not been established
  - The high number of oocytes obtained via OPU seems to be a unique characteristic of Nelore cows. However, are similar to other *B. indicus* and *B. taurus* breeds when comparing the average embryo production by MOET



Comparison of embryo yield and pregnancy rate between *in vivo* and *in vitro* methods in the same Nelore (*Bos indicus*) donor cows

J.H.F. Pontes<sup>a</sup>, I. Nonato-Junior<sup>a</sup>, B.V. Sanchez<sup>a</sup>, J.C. Ereno-Junior<sup>a</sup>, S. Uvo<sup>a</sup>, T.R.R. Barreiros<sup>b</sup>, J.A. Oliveira<sup>a</sup>, J.F. Hasler<sup>a</sup>, M.M. Seneda<sup>b,c,d</sup> Theriogenology 71 (2009) 690–697

Variation in embryo production among 6 Nelore cows (I–VI), comparing *in vitro* (OPU/IVF) versus *in vivo* (MOET) procedures.

|                                    | Donors (I–VI) |      |      |      |      |      |
|------------------------------------|---------------|------|------|------|------|------|
|                                    | I             | II   | III  | IV   | V    | VI   |
| Total no. OPU IVF                  | 5             | 5    | 4    | 4    | 5    | 5    |
| Mean no. oocytes/collection        | 36.6          | 25.6 | 49   | 29.7 | 22.8 | 16   |
| Mean no. viable oocytes/collection | 32.2          | 23.4 | 45.2 | 26   | 19.6 | 14.4 |
| Mean no. embryos/OPU IVF           | 15.6          | 10.4 | 24.1 | 10.3 | 6.8  | 3.8  |
| Mean no. pregnancies/OPU IVF       | 4.8           | 2.8  | 9.25 | 4.3  | 2.2  | 1    |
| Total no. MOET                     | 2             | 3    | 2    | 2    | 2    | 3    |
| Mean no. embryos/collection        | 10            | 4.3  | 6.5  | 2    | 12.5 | 5.3  |
| Mean no. pregnant/collection       | 5.5           | 2    | 1    | 1.5  | 6.5  | 1.3  |



### OPU equipment: ECM-IMV

**OVUM PICK UP**

**«ALL IN ONE» TRANSDUCER FOR BOVINE AND EQUINE**

The ECM OPU probe is the result of field request, analysis followed by development and testing by leading veterinarians and technicians in the field of IV and OPU. Our OPU transducer makes ultrasound ovum aspiration easier and more comfortable due to its size, lightweight and field of view.

**FEATURES**

- Slim design, ideal to be used on young heifers and mares
- All metal needle guide and needle bushing accurate guidance
- Ready to use, easy and quick assembly - time saving between animals
- Instant needle visualization, needle track in the middle of the screen (graduated theory line) (8mm follicles visualization)
- Compatible with short and long needles
- Usable on many species: bovine, equine, wild if animals...
- Easy and fast cleaning and disinfection

**DATA SHEET / SPECIFICATIONS**

- Frequency: wideband 6,5 Mhz (B Image 1,5,0 - 6,5 - 7,5 Mhz)
- No of elements: 128
- Pitch: 0,209mm
- ROC (radius of curvature): 10mm
- Ovu. Trans: 8mm
- Focus lens: 35mm
- FOV (field of view): 150°
- Length of guide: 61,5 cm
- Weight: less than 1 kg
- Length of cable: 200 cm
- Bopsy guide: graduated line (mm) orientation of image (Rightdown - Left/Right)
- Compatibility: EXAGO & EXAPAD

**EXAGO**

**EXAPad**

100% MADE IN FRANCE  
WWW.ECMSCAN.COM

**ECM**  
ECHO CONTROL MEDICAL





## "All in one" transducer (ECM-IMV)



## OPU equipment: MINITUBE (Bovine)

### ASPIRACIÓN DE OVOCITOS



#### SOLUCIONES MINITUBE PARA UNA COLECCIÓN EXITOSA DE OVOCITOS

##### Porta-sonda de ultrasonido

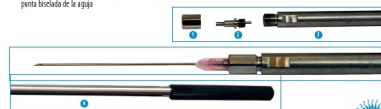
- Diseño delgado permite la aspiración de ovocitos en hembras jóvenes (especialmente en combinación con una sonda portátil)
- Saldas optimizadas para aguas recomendadas (23360/1200 y 23360/1250)
- Sin riesgo de desprendimiento de la aguja
- El material se calienta muy rápidamente a la temperatura corporal
- Punta-sonda muy ligera y suave, fácil de manejar
- Incluye dispositivo para aguja y maletín de transporte

El soporte de sonda está disponible para calzar con GE, SonoSite, Aloka, RCI o sonda ECM. Bajo pedido también se puede adaptar el dispositivo a otros sondas.

|          |                   |
|----------|-------------------|
| GE       | ref. : 23400/0120 |
| SonoSite | ref. : 23400/0040 |
| Aloka    | ref. : 23360/0600 |
| RCI      | ref. : 23400/0115 |
| ECM      | ref. : 23400/0413 |



- La unidad de aguja consiste en:
- Tuercas de sujeción para la fijación segura de la aguja: proporciona una fijación segura de la aguja en su posición, no permitiendo ningún espacio muerto entre la aguja y el adaptador
  - El adaptador de la aguja: conecta la aguja con el tubo
  - Tubo de conexión de acero inoxidable
  - Los mangos de forma redonda: permiten un posicionamiento variable de la punta biselada de la aguja



www.minitube.com



##### Aguja de aspiración desechable

- Fácil y económica de reemplazar
- Agua nueva para cada sesión de OPU → alto estándar higiénico
- Corta y fácil de manejar
- 100 agujas / empaque

|                              |                   |
|------------------------------|-------------------|
| Para vacas (1,2 x 75 mm)     | ref. : 23360/1200 |
| Para vaquillas (0,8 x 70 mm) | ref. : 23360/1250 |

##### Set de mangueras

- Tubo de material firme
- El conector ovocito-camilla no se adhiere a las paredes
- Con lock para una conexión a prueba de fugas con el tapón de goma
- Estéril y envasado individualmente

|                  |                   |
|------------------|-------------------|
| Set de mangueras | ref. : 23360/1000 |
|------------------|-------------------|

##### Accesorios

- Tapón de goma: para la conexión con tubo de 50 ml (1 empaque)
- Presección para partesanas, desechable (44 unidades/bolsa)
- Gel para examen ultrasonico (250 g)
- Tubo de colección, 50 ml

|                             |                   |
|-----------------------------|-------------------|
| Tapón de goma               | ref. : 23360/1100 |
| Presección para partesanas  | ref. : 23360/1260 |
| Gel para examen ultrasonico | ref. : 23400/0500 |
| Tubo de colección           | ref. : 17220/0017 |

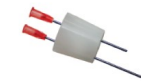
##### Bomba de aspiración OPU

- Con calentador de medios
- Transferencia de vacío a la aguja extremadamente rápida
- Operada mediante pedal
- Aspiración ajustable hasta 275 mm Hg
- Temperatura de calentamiento ajustable hasta +50°C

|                            |                   |
|----------------------------|-------------------|
| Bomba de aspiración, 240 V | ref. : 23362/0000 |
| Bomba de aspiración, 120 V | ref. : 23362/0001 |

\*Colección de ovocitos = Ovario Pick-Up = OPU

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## OPU equipment: MINITUBE (Equine)



### RECOLECCIÓN DE OVOCITOS EQUINOS

**PORTA SONDAS Y AGUJA DE DOBLE LUMEN PARA LA ASPIRACIÓN TRANSVAGINAL DE FOLÍCULOS (OPU)**

**Descripción**

- El porta sonda tiene incluida una guía para la aguja.
- El porta sonda se utiliza con una aguja de doble lumen; ambos espacios permiten la aspiración simultánea o la aspiración alternada con inyección de fluidos hacia o desde los folículos.
- La aguja de recolección OPU es conectada a la bomba de aspiración de ovocitos equinos (Ref. 23362/0002 (230 V) o 23362/0003 (115 V)).

**Sus beneficios**

- El diseño delgado y ergonómico de la sonda proporciona un fácil manejo.
- El porta sonda se temple rigidamente a la temperatura corporal.
- Un mango desmontable puede acoplarse a ambos lados si fuera necesario.
- El porta sonda se suministra dentro de una caja de transporte sólida.
- Saldos optimizados de la aguja de doble lumen permite un excelente control de la aguja.
- Punta de la aguja cóncava.
- Los conectores estériles de la aguja son herméticos, permitiendo el paso seguro de los ovocitos.

**Referencias**

|   |                   |
|---|-------------------|
| Portador de sonda de ecógrafo para sonda GE | Ref. : 19009/2100 |
| para sonda Esaote                           | Ref. : 19009/2101 |
| Aguja, 12, 5', 25', sistema de doble luz    | Ref. : 19009/2105 |
| Tubos desechables                           | Ref. : 19009/4101 |

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### BOMBA OPU PARA ASPIRACIÓN DE FOLÍCULOS

Las bombas y un bloque de calentamiento han sido combinados en una unidad para la aspiración eficiente de ovocitos equinos. El dispositivo es utilizado para aspirar fluido folicular de folículos equinos e inyectar en medio a estos folículos. La inyección del medio puede efectuarse en forma simultánea o alternada con la aspiración. El procedimiento de lavado repetido de los folículos conduce a una elevación sustancial de las tasas de recuperación de ovocitos equinos. El nivel de presión y la histéresis son programables por el usuario. El control de la bomba es mediante un pedal. El medio de lavado y colección es calentado en un bloque templado que ofrece espacio para dos bombas de 250 ml y espacio adicional para cuatro tubos de 50 ml. Los ovocitos son aspirados directamente a una botella llenada con medio pre-templado. Los ovocitos se almacenan en el medio dentro de un rango de temperatura óptimo para los ovocitos (recomendamos +9°C a +18°C). La temperatura de régimen está ajustada a +37°C.

**Datos técnicos**

- Voltaje de operación: 220-240 V~, 50-60 Hz; también disponible para 115 V.
- Temperatura de entorno: +5°C hasta +45°C.
- Dimensiones: 430 x 350 x 220 mm (Ancho x Profundidad x Alto).
- Peso: aprox. 14 kg.
- Presión relativa negativa:  $P_{rel}$  = máx. -300 mmHg.
- Presión máx. aprox. 750 mm Hg.

**Referencias**



Bomba OPU para aspiración de folículos en equinos Ref. : 23362/0002




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## OPU equipment



- Ultrasound scanner (FalcoVET)
- Transvaginal probe (R-10, 5-7.5 MHz)
- OPU "handgrip" (60 cm)
- Punction guide
- Disposable puncton needles (18 G, 1.2 x 40 mm)





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### OPU equipment






- Ultrasound scanner (GE, Voluson 730 P)
- Transvaginal probe (IC5-9, 5-9 MHz)
- OPU "handgrip" (60 cm)



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### OPU equipment

- Vacuum pump (70 mm Hg) (footpedal)
- Water bath (38°C)
- Probe cover (ultrasound gel)
- PBS (heparine 0.01%, FCS 1%)



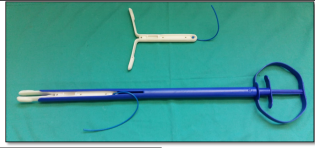



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**PROTOCOLO ESTIMULACIÓN OVÁRICA PARA DEMO OPU DIA 14 DIC**  
**(DEMO OPU Santarem)**

| FECHA                | MAÑANA (8:00)  | TARDE (20:00)   |
|----------------------|--|---|
| Lunes 2 DIC          | Poner CIDR* (Zoetis)<br>GnRH (Cystoreline, Ceva)<br>4 ml |   |
| Sábado 7 DIC         |  | PGF2a (Dinolityc, Zoetis)<br>5 ml<br><br>Retirar CIDR |
| Domingo 8 DIC        |  | PGF2a (Dinolityc)<br>5 ml                             |
| <u>Martes 10 DIC</u> | GnRH (Cystoreline)<br>4 ml                               |   |
| Miércoles 11 DIC     |  | FSH** (Pluset, Calier)<br>2.5 ml                      |
| Jueves 12 DIC        | FSH (Pluset)<br>2 ml                                     | FSH (Pluset)<br>1.5 ml                                |
| Viernes 13 DIC       | FSH (Pluset)<br>1 ml                                     |   |
| <u>Sábado 14 DIC</u> | <u>OPU</u>   |   |

**Observaciones:**

- \*CIDR es opcional. Si NO pones CIDR, el protocolo empieza **Martes 10**.
- \*\*FSH (Pluset, Calier): 7 ml por vaca (350 UI) repartidas en 4 inyecciones. 1 caja de Pluset lleva 2 viales de 500 UI y un diluyente de 21 ml (para 3 vacas).

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- **Donor preparation**
  - Restrain and waxing, Rectum cleaning
  - Perineal area and vulva cleaning
  - Palpation and rectal examination
  - Revision and Fix ovaries
- **Drugs treatment pre-OPU**
  - Analgesia: carprofen (Rimadyl, 10-12 ml, s.c.)
  - Tranquilization: xylazine 2% (Rompun or Nerfasin, 0.6-0.9 ml, i.m.)
  - Epidural anesthesia: lidocaine (Anesvet, 3-6 ml)



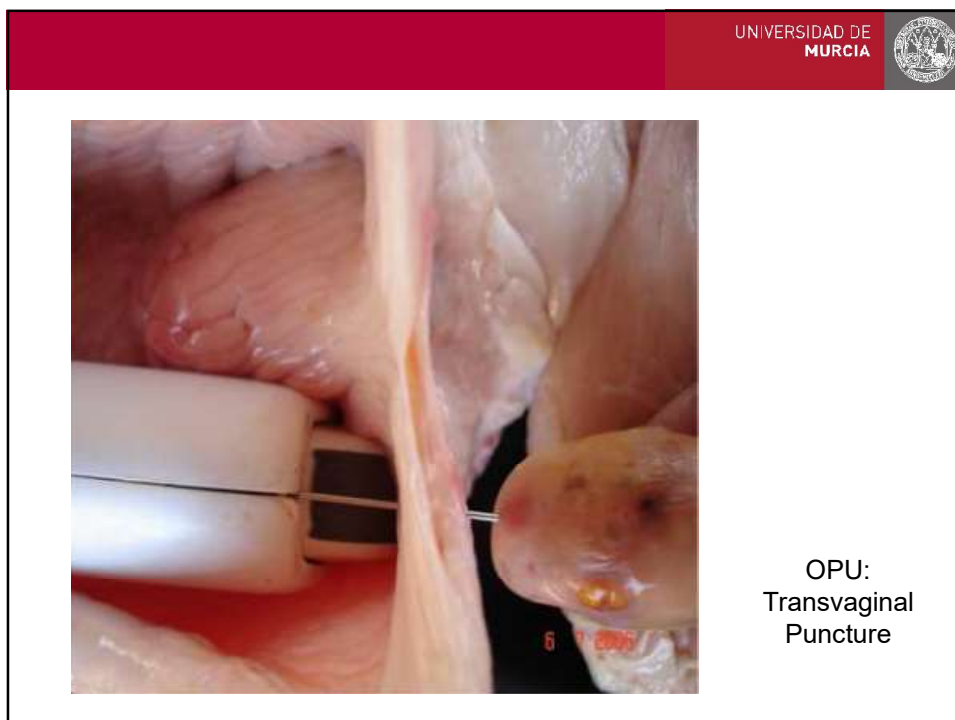
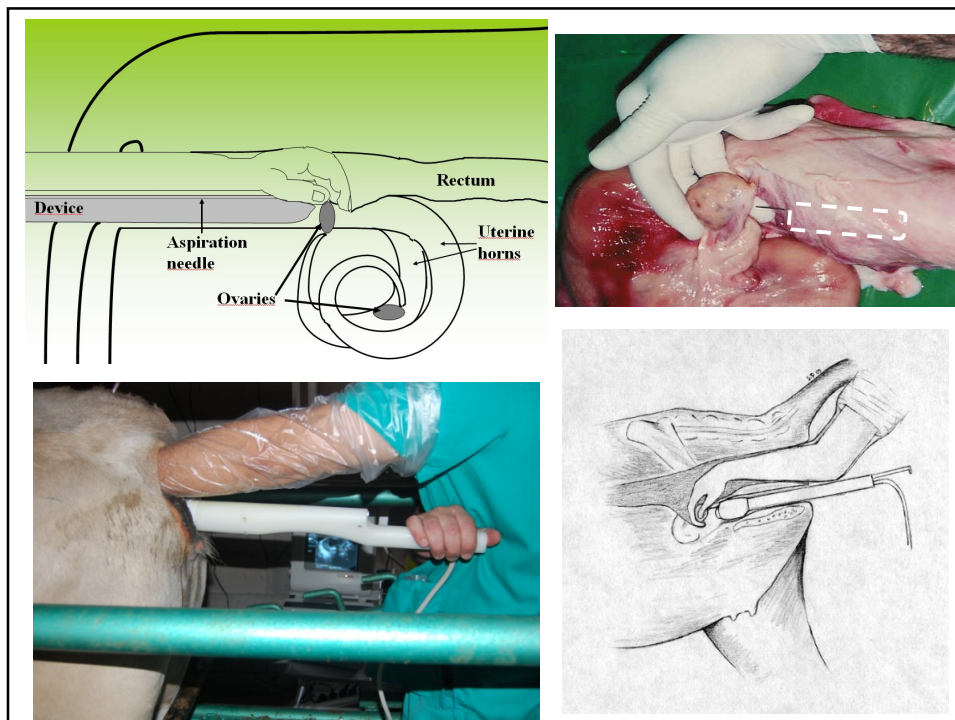




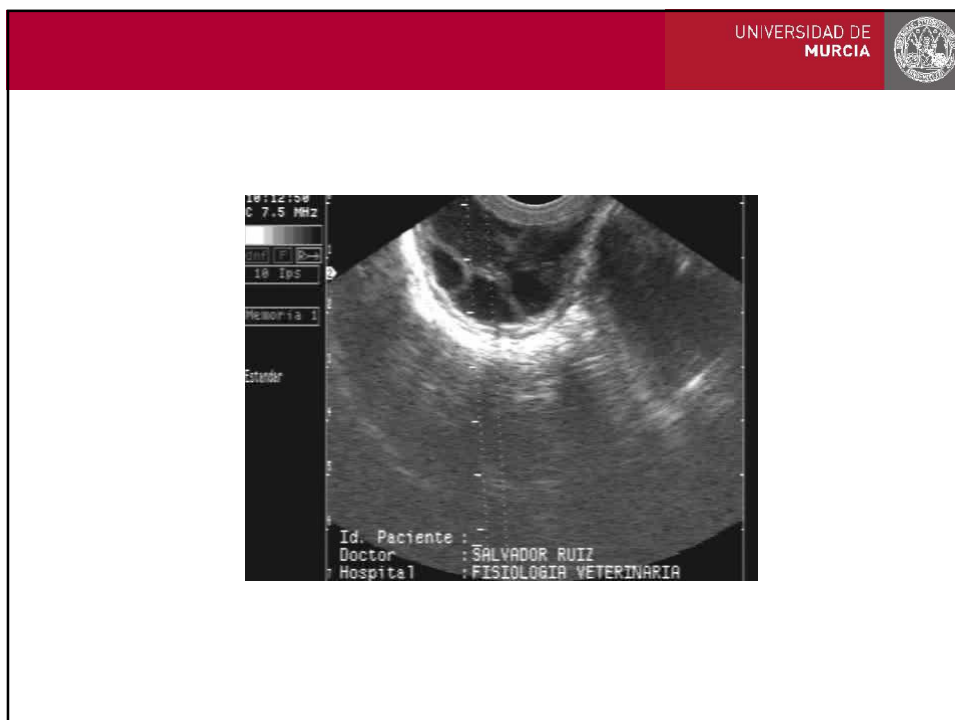
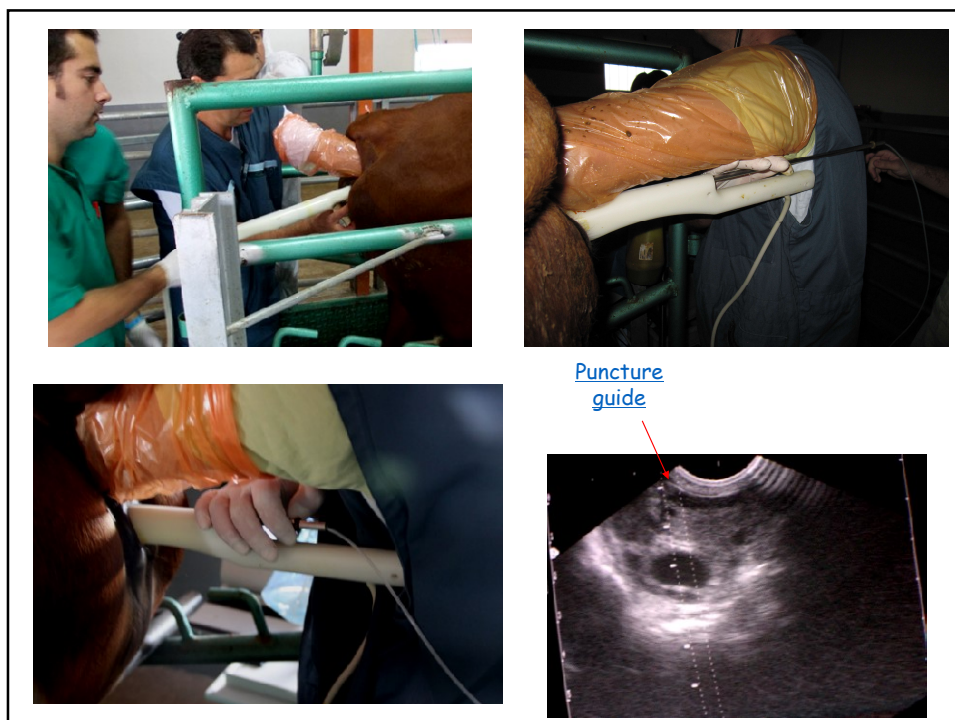
**Ovum Pick-Up (OPU): Alternativa emergente para a produção de embriões**  
Salvador Ruiz



**Ovum Pick-Up (OPU): Alternativa emergente para a produção de embriões**  
Salvador Ruiz

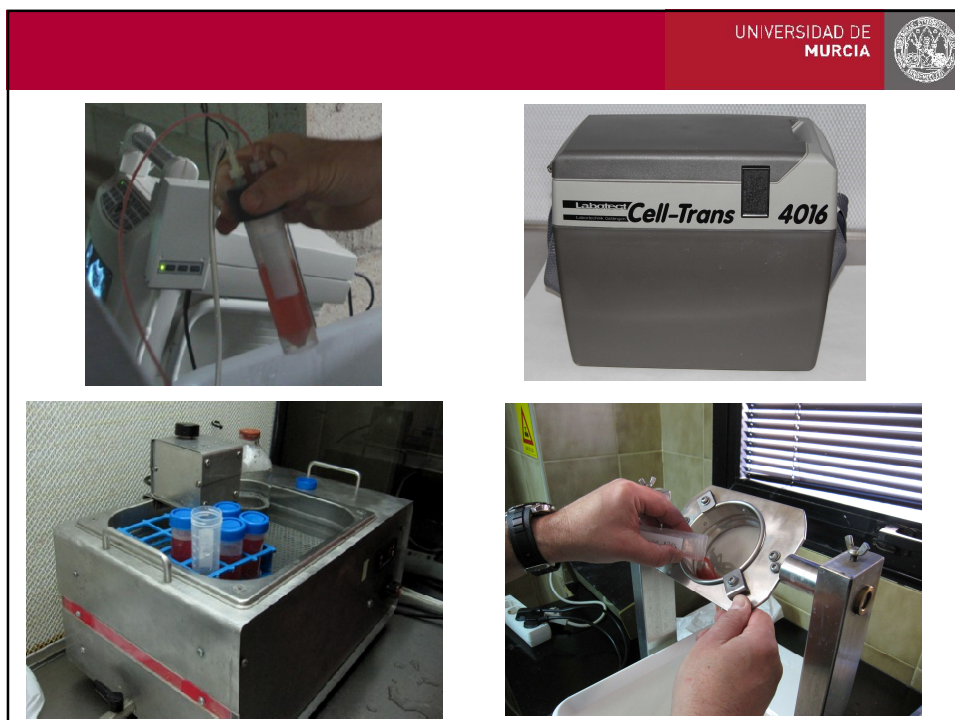


**Ovum Pick-Up (OPU): Alternativa emergente para a produção de embriões**  
Salvador Ruiz



# Ovum Pick-Up (OPU): Alternativa emergente para a produção de embriões

Salvador Ruiz

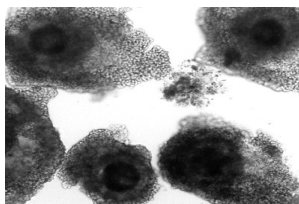






### CCOs classification

(Oropeza et al., 2004)



Type I oocytes (very good quality):

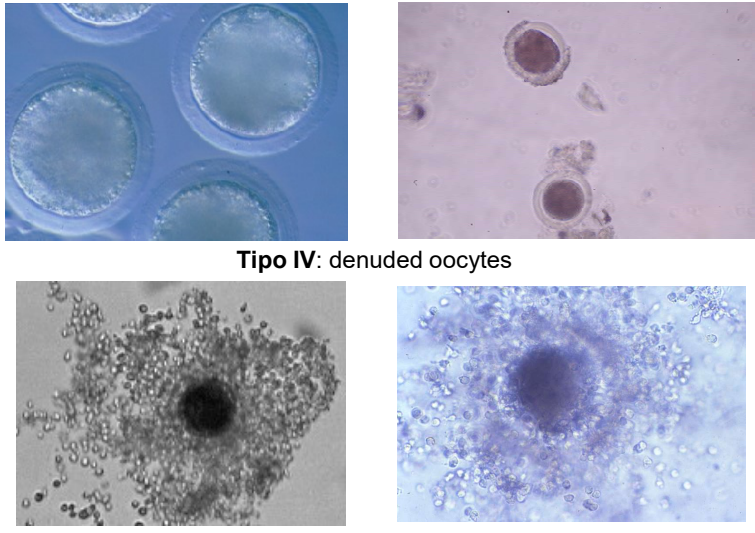
- Dark and homogeneous ooplasm
- Many layers of *cumulus* cells

Types II and III oocytes (good and intermediate quality): Homogeneous, clear or pigmented ooplasm. Fewer layers *cumulus* cells



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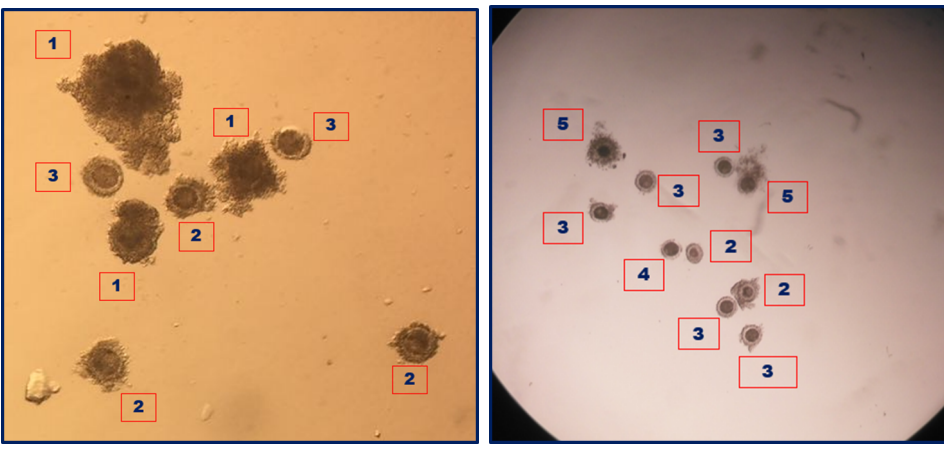
### Not suitable oocytes in OPU/IVEP

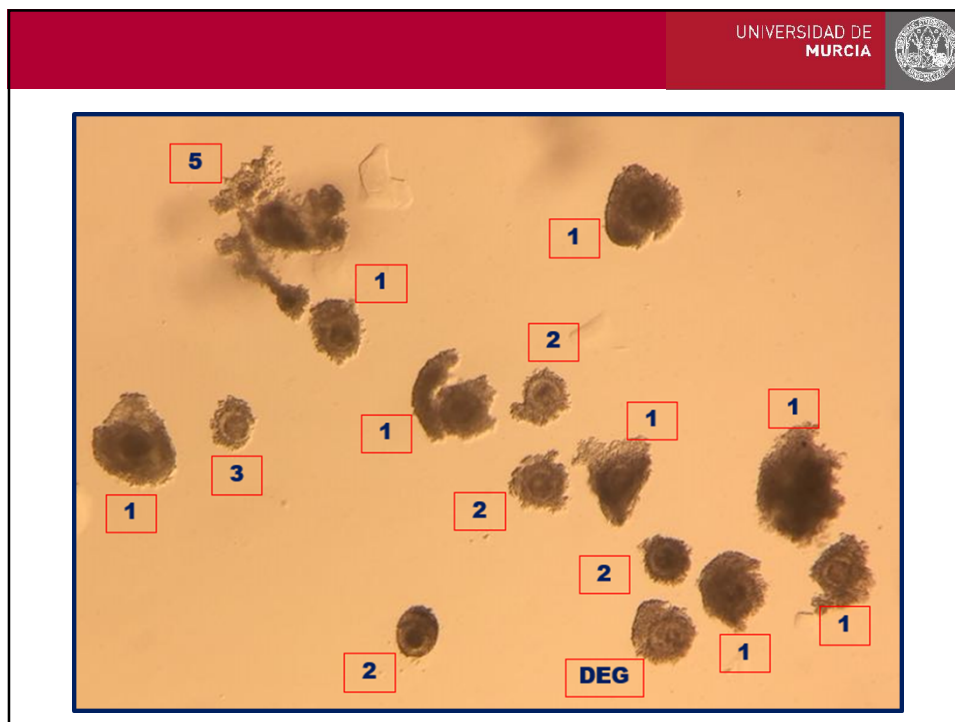


**Tipo IV:** denuded oocytes

**Tipo V:** *in vivo* matured oocytes

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### OVUM PICK-UP (OPU) & IN VITRO EMBRYO PRODUCTION (IVEP)

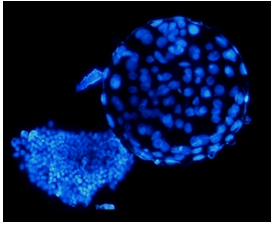


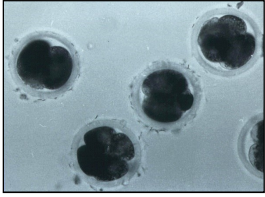

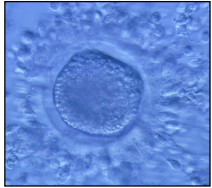
- IVEP steps:
  - *In Vitro* Maturation (IVM)
  - *In Vitro* Fertilization (IVF)
  - *In Vitro* Culture (IVC)

The slide contains several images illustrating the IVEP process: a laboratory with incubators, a pipette, a microscopic view of oocytes, a microscopic view of a single oocyte, a microscopic view of oocytes with blue fluorescence, and a microscopic view of oocytes in petri dishes.

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### IVEP


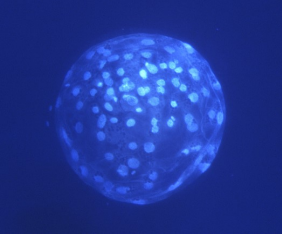


- **IVM:** TCM-199 (24 h, 38.5°C, 5%CO<sub>2</sub>)
- **Semen:** *Swim-up*. Density Gradients (Percoll®, Bovipure®) 1 x 10<sup>6</sup> spz/ml
- **IVF:** SPERM-TALP & FIV-TALP (22h, incubation)
- **IVC:** SOF + 5% FCS (38.5°C, 5%CO<sub>2</sub>, 5%O<sub>2</sub>)
- Cleavage: 48 h
- Blastocysts: 7 d
- IVEP Efficiency:
  - IVM: 90%
  - IVF: 80%
  - IVC: 35-40%



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### OPU/IVEP yield

- IVM: 90%
- IVF: 80%
- Blastocysts rate (IVC): 35-40%
- Pregnancy rate after ET: < 50%
- 10 -15 offspring/100 oocytes in culture



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### Other uses of OPU in cattle

- Tool for interfering with follicle dynamics to investigate its regulatory mechanisms
- Eliminate the deleterious effect of a dominant follicle during superovulation in cows, by puncturing the dominant follicle 38-46 h prior to superovulatory treatment
- Therapeutic effect on infertile donors, especially affected by ovarian cysts. Safe and good method for the manual, active rupturing of cysts during rectal palpation
- Source of oocytes for studies of IVF, IVEP, clonning and transgenesis. Also, in ARTs for the recovery and conservation of endangered bovine breeds
- Other uses of OPU: injections (ovarian intrafollicular, ovarian stroma, intraluteal). Biopsies (CL and ovarian stroma for primordial and preantral follicles)

**Ovarian injection**

**Ovarian biopsy**

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### OPU in other species

- **OPU in Buffaloes:** some countries interested in reproduction biotechnologies in buffaloes (Italy, Argentina, China, India). OPU has great potential because MOET programs have given poor results compared with cattle. Ovaries are small and the follicles tend to be fewer and of small diameter. OPU with sexed semen and cryopreservation accelerates genetic gain in buffalo industry
- **OPU in Equine:** using a double-lumen 12G needle. In preovulatory follicle (> 35 mm) oocytes can be collected, 24 h after hCG with the donor showing signs of uterine edema. For OPU equine immature oocytes, aspirate follicles (8-10 follicles >1 cm) preferably without dominant follicle (transition season). Oocyte recovery rates from immature follicles is low because large size of follicles and the strong attachment to the follicle wall of COCs. It is necessary to use double lumen needles for repeated flushing, up to 8 to 10 times for each follicle

Embryo production by ovum pick up (OPU) in Mediterranean Buffalo (Galli et al. unpublished). C. Galli et al. / Theriogenology 81 (2014) 138-151

| No. of OPU | No. of follicles | No. of oocytes | No. cleaved | Cleavage (%) | No. of embryos | No. of embryos per OPU | % embryos/ oocytes | % embryos/ cleaved |
|------------|------------------|----------------|-------------|--------------|----------------|------------------------|--------------------|--------------------|
| 123        | 1392             | 815            | 389         | 47.73        | 132            | 1.07                   | 16.20              | 33.93              |

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Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice  
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