



Transferencia de conocimientos

PROGRAMA DE VINCULACIÓN DE CIENTÍFICOS Y TECNÓLOGOS

Dra. Johanna Leiva Revilla



UNIVERSIDAD PERUANA
CAYETANO HEREDIA
FACULTAD DE CIENCIAS Y FILOSOFÍA



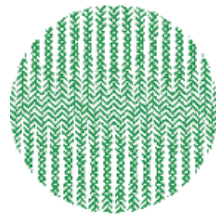
UNIVERSIDADE
ESTADUAL DO CEARÁ



PROCIENCIA
PROGRAMA PARAGUAYO PARA EL DESARROLLO DE LA CIENCIA Y TECNOLOGÍA



UNIVERSIDAD DE
MURCIA



INRA

Institut National de la Recherche Agronomique

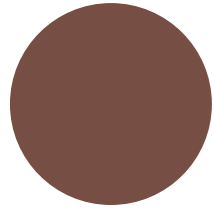
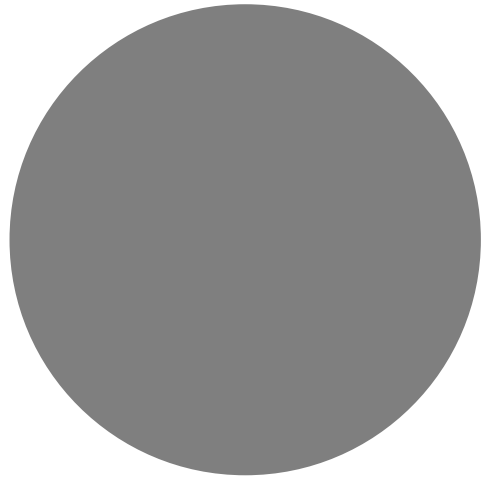




Biotecnología de la reproducción

- La biotecnología tiene un gran impacto en la mejora de la raza, la tasa reproductiva y la producción animal
- Las aplicaciones reproductivas más comunes que se integran con la biotecnología son:
 - Inseminación artificial (AI)
 - Superovulación
 - Análisis de calidad seminal
 - Sexado de espermatozoides (SPZ)
 - Transferencia de embriones (ET)
 - Producción in vitro de embriones (IVEP)
 - Criopreservación de gametos y embriones
 - Manipulación in vitro de ovocitos incluidos en folículos ováricos pre-antrales (MOIFOPA)





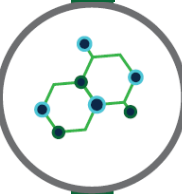
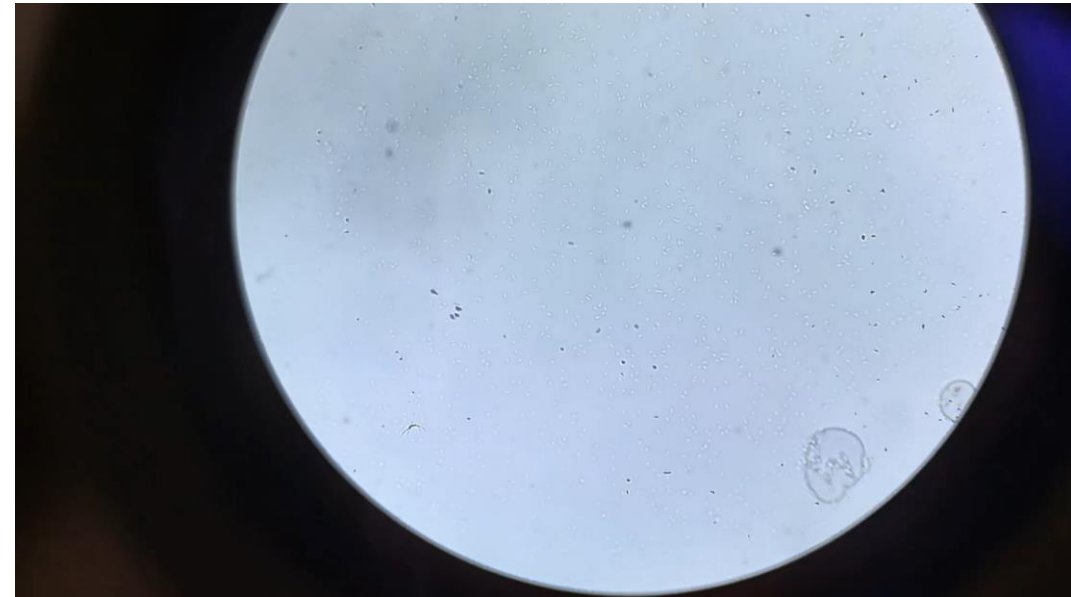
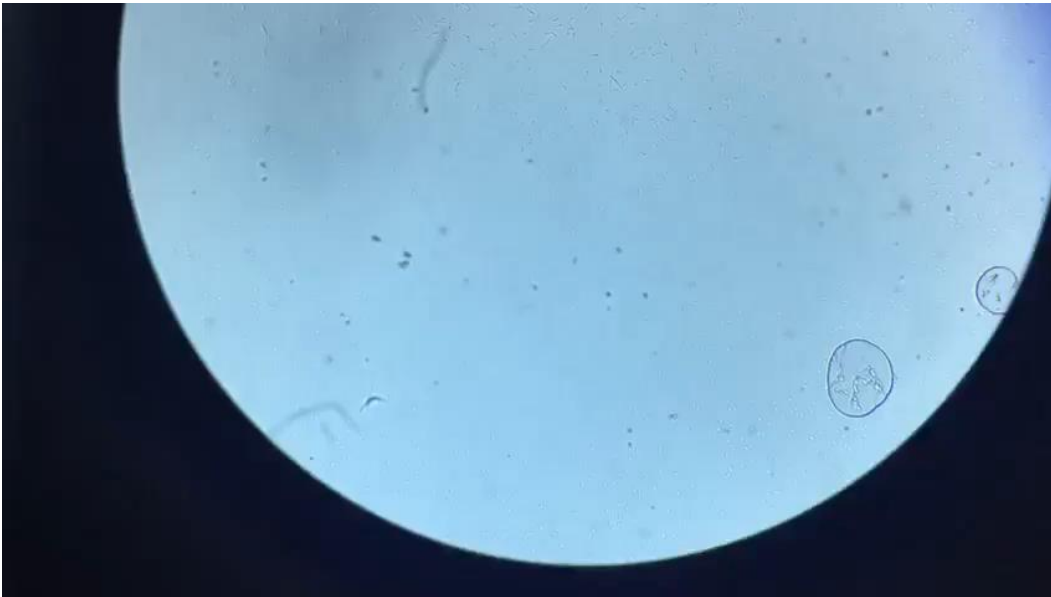
Análisis de la calidad seminal



Análisis de la calidad seminal en cerdos

Espermatograma (espermiograma) normal:

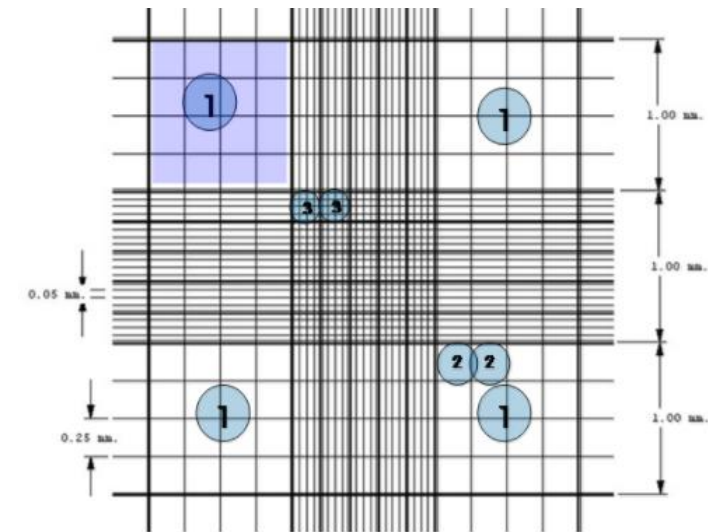
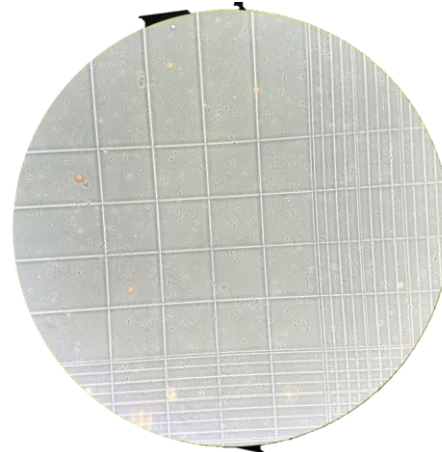
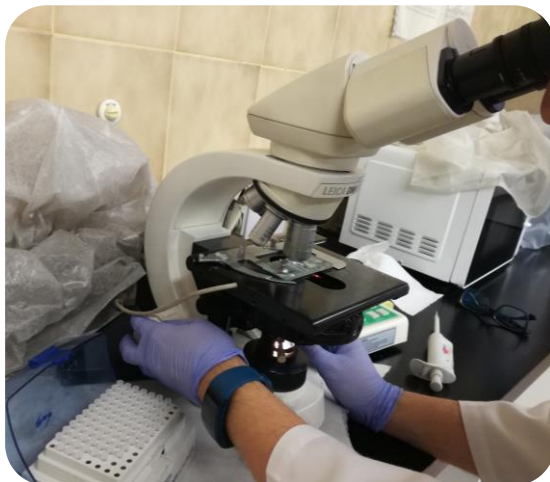
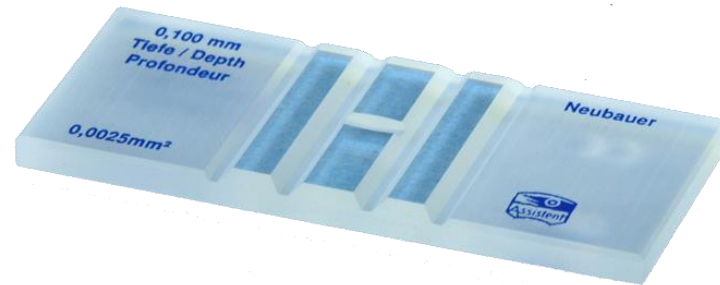
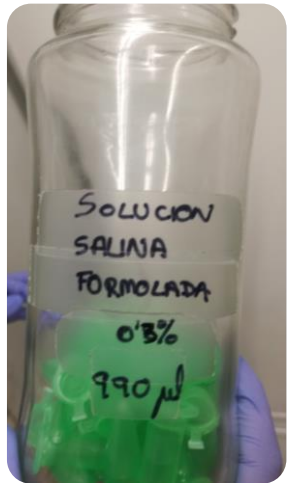
- Análisis rápido en microscopio a 37° C



Análisis de la calidad seminal en cerdos

Espermatograma (espermiograma) normal:

- Concentración



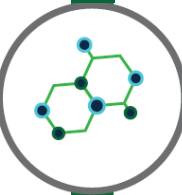
Cuadrado 1

Área = 1 mm x 1mm = 1 mm²

Volumen = 1 mm² x 0,1 mm = 0,1 mm³ = 1 x 10⁻⁴ ml

1

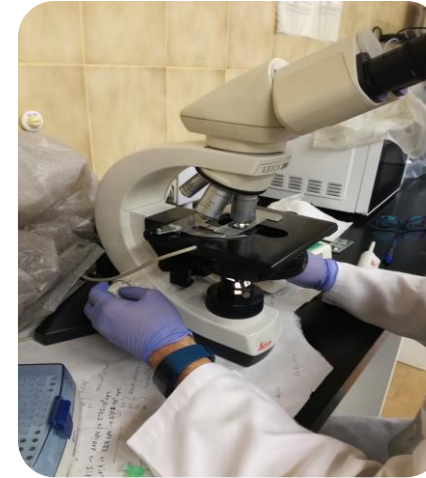
$$\text{Concentración} = \frac{\text{Total Células Contadas} \times 10.000}{\text{Número de Cuadrados}}$$



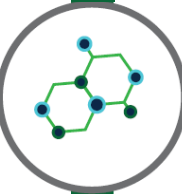
Análisis de la calidad seminal en cerdos

Espermatograma (espermiograma) normal:

- Morfología



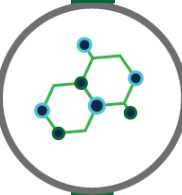
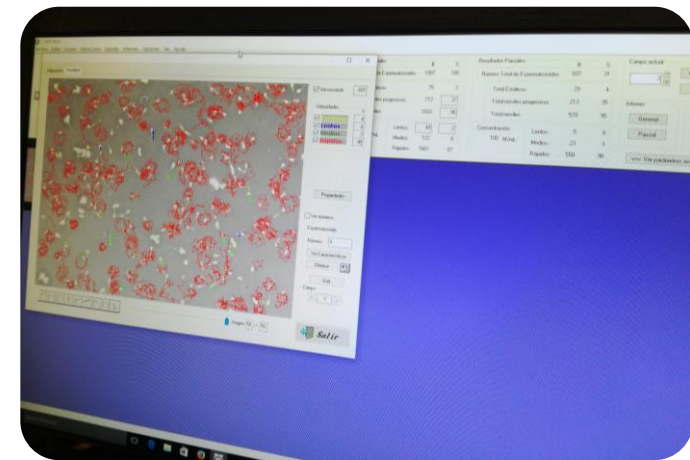
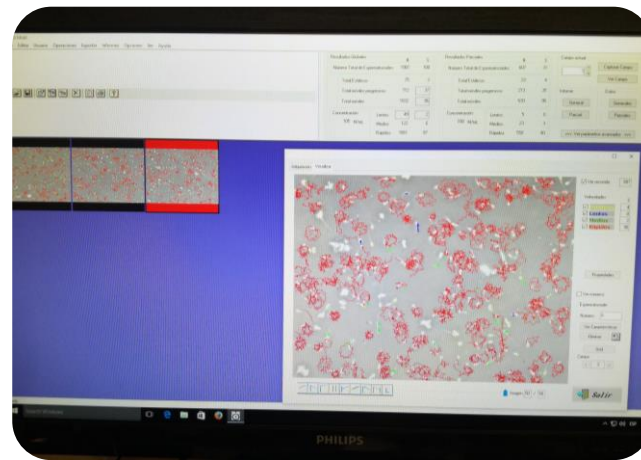
Normal	Cabeza grande	Pieza intermedia	Cola látigo	Cola enroscada	Gota citoplasmática distal	Gota citoplasmática proximal	Otros



Análisis de la calidad seminal en cerdos

Espermatograma (espermiograma) normal:

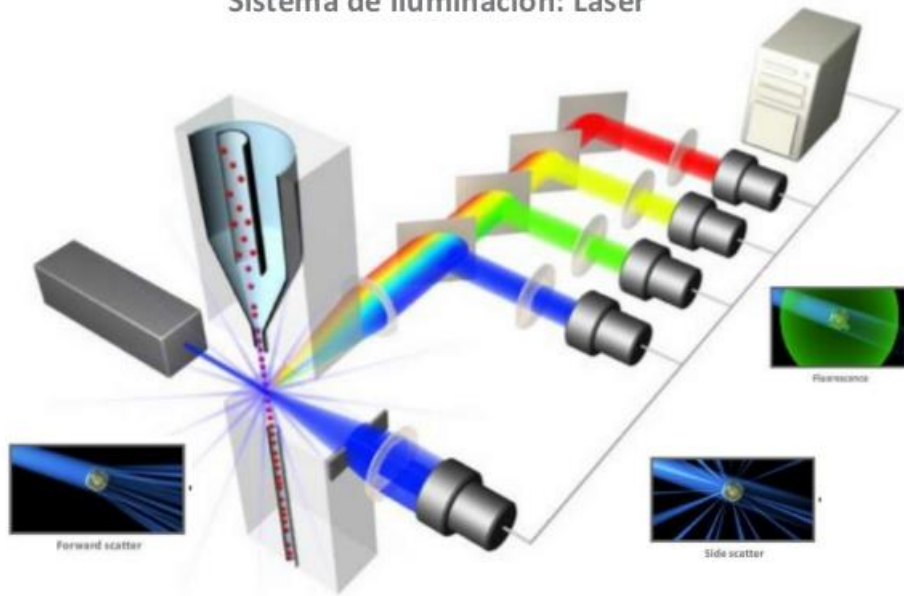
- Motilidad - Sistema CASA
 - Motilidad
 - Rápido, medio, no progresivo, no motil
 - Velocidad y trayectoria



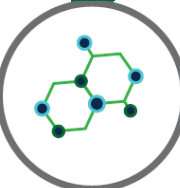
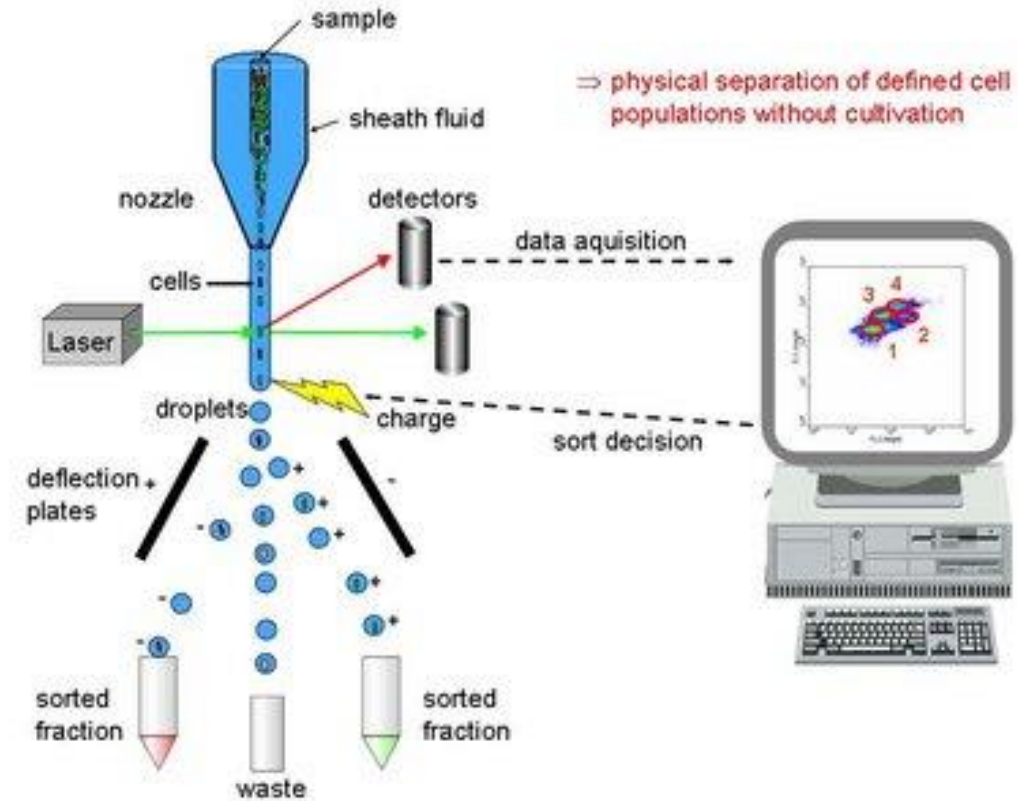
Análisis por citometría de flujo



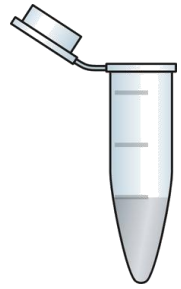
Sistema de iluminación: Láser



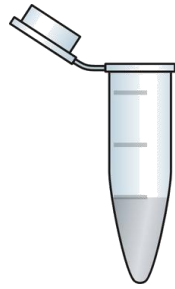
Izasa Scientific
A VWR Group Company



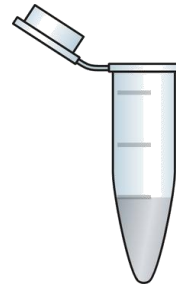
Análisis por citometría de flujo



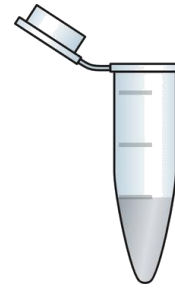
Control sin muestra



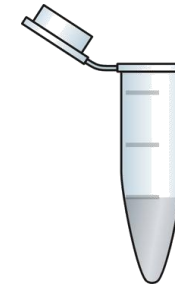
Control con muestra



Muestra + PI



Muestra + H2DCFDA



Muestra + MIX

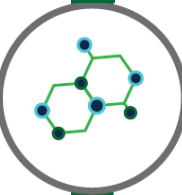
1 mL de muestra [$1-10 \times 10^6 \times \text{mL}$] + 5 uL de solución stock de PI [5 uM]

1 mL de muestra [$1-10 \times 10^6 \times \text{mL}$] + 5 uL de solución stock de H2DCFDA [5 uM]

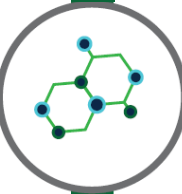
Incubación por 15 min a **37°C**



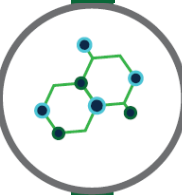
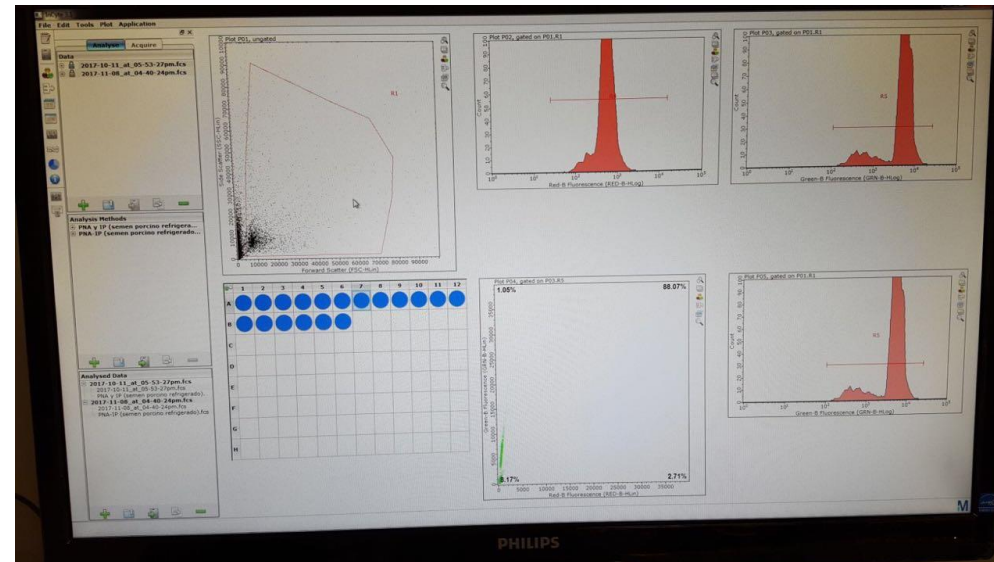
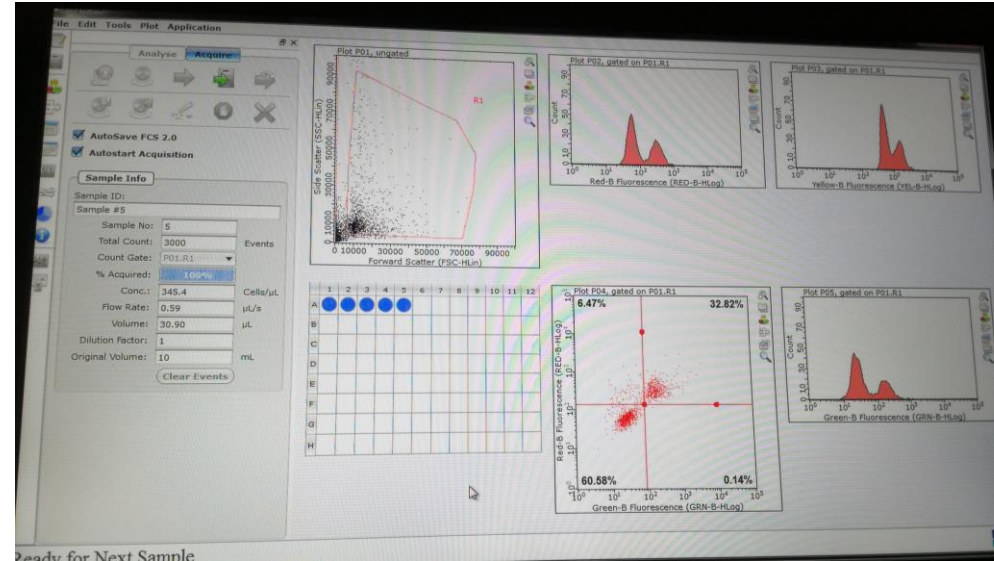
Yoduro de propidio (PI)
2'7'-dichlorodihydrofluorescein diacetate (H2DCFDA)
PI + H2DCFDA (MIX)



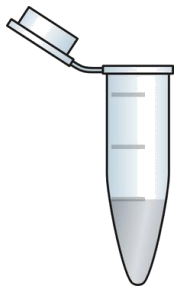
Análisis por citometría de flujo



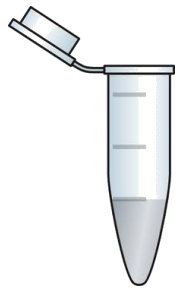
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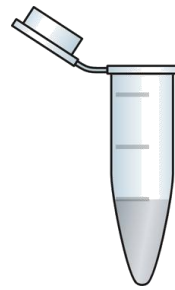
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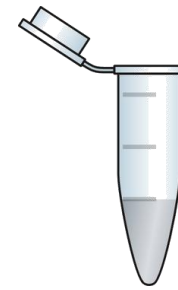
Control sin muestra



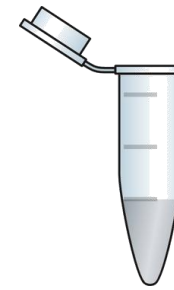
Control con muestra



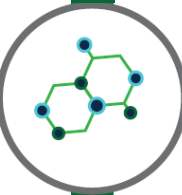
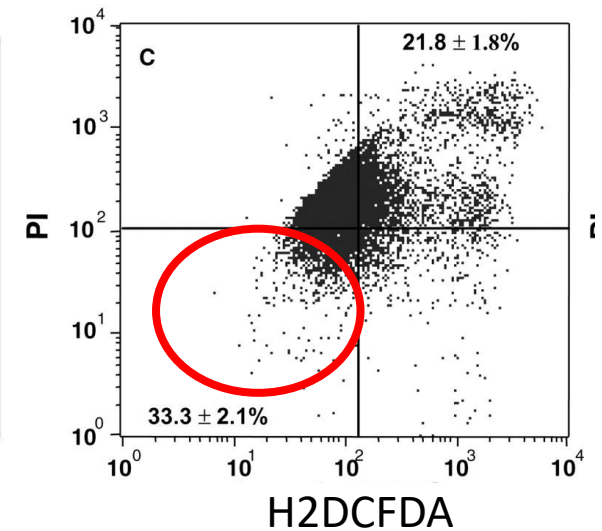
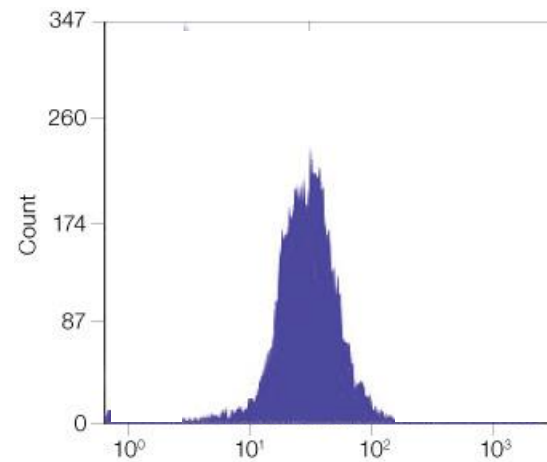
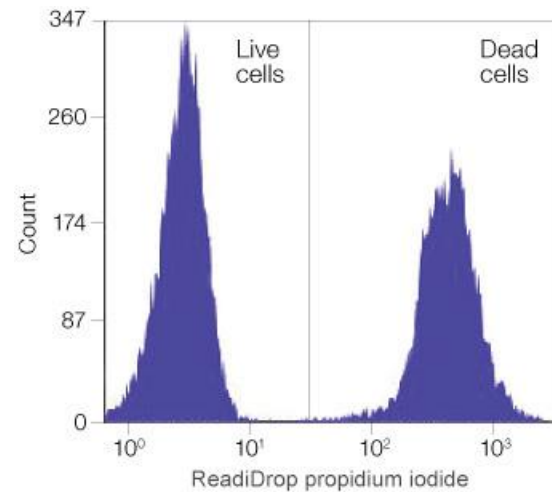
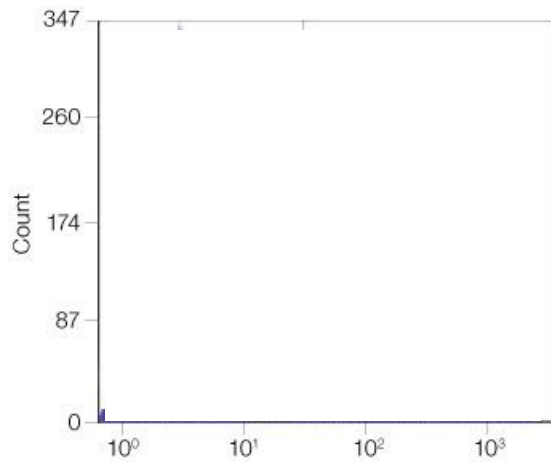
Muestra + PI



Muestra + H2DCFDA

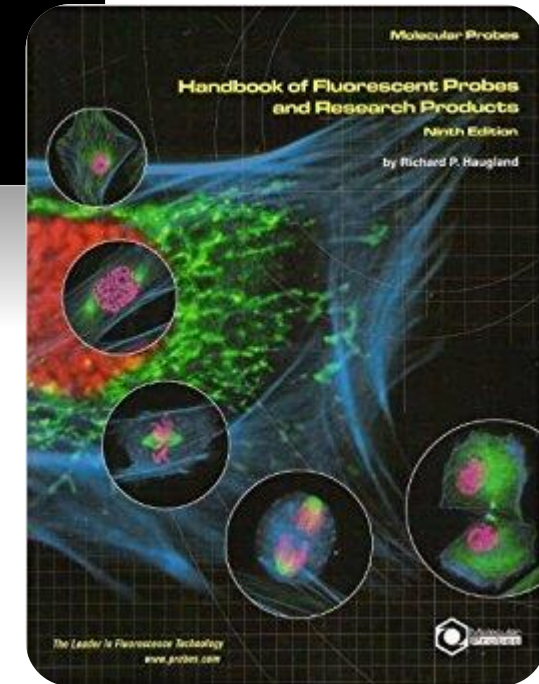


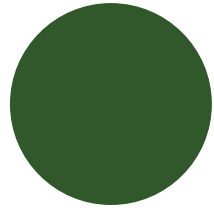
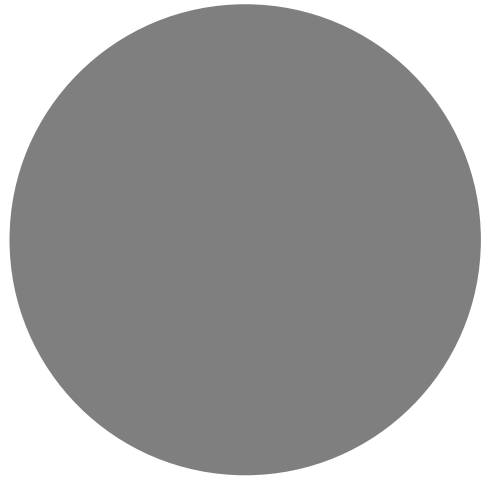
Muestra + MIX



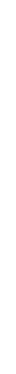
Análisis por citometría de flujo

- Viabilidad
- ROS
- Calcio
- Fragmentación de ADN
- Desorden lipídico



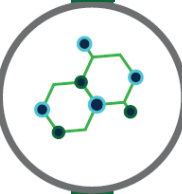


Producción in vitro de embriones



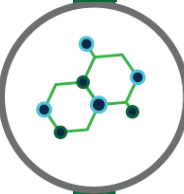
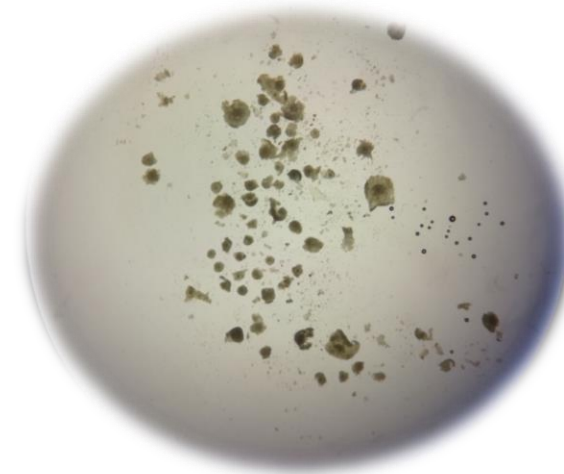
Colecta de ovarios

- Colecta de ovarios de matadero local



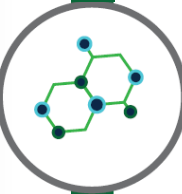
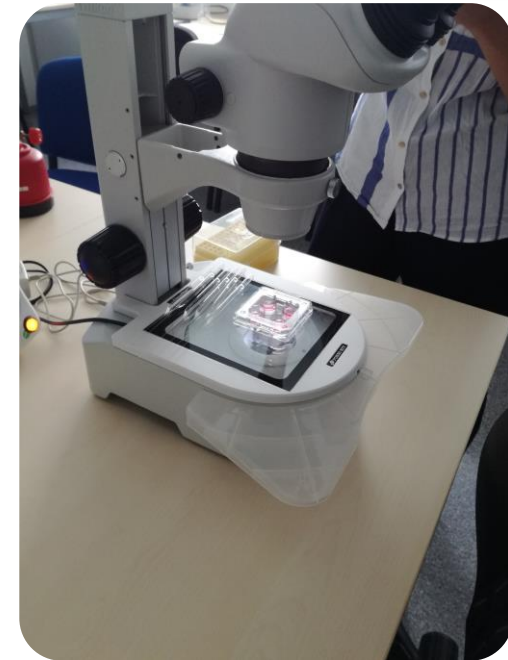
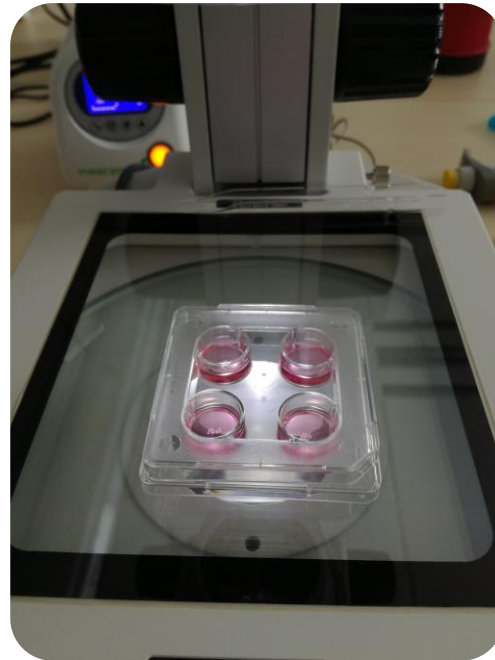
Colecta de complejo-cumulus-oophorus (CCOs)

- Lavado de ovarios
- Punción de ovarios
- Pesca de CCOs



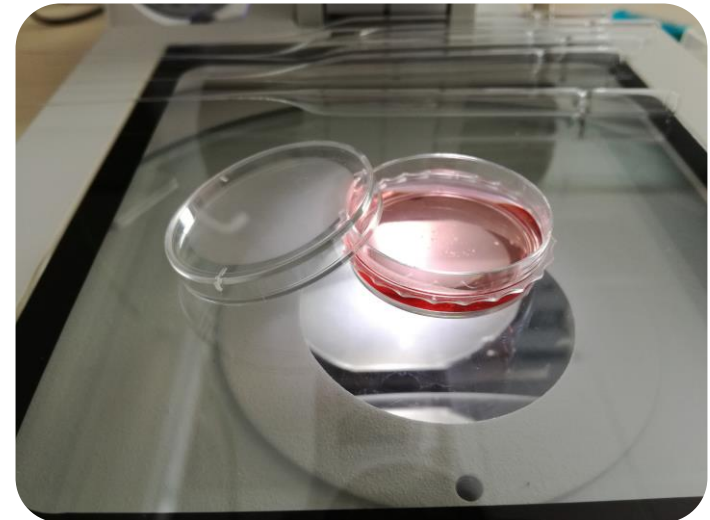
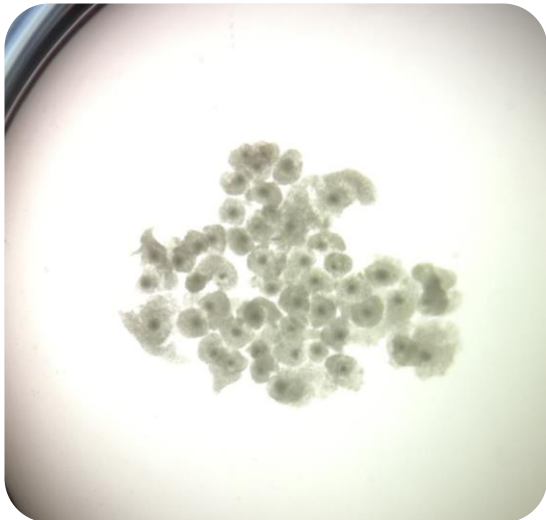
Maduración in vitro de CCOs

- Seleccionar CCOs de buena calidad
- Colocar en placas (grupos de 50) con medio IVM
- Incubar por 24 hrs



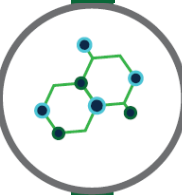
Preparación de ovocitos maduros

- Analizar expansión del cumulus
- Lavar con medio IVF-TALP+
- Colocar en placas de 4 pozos con 250uL de medio



Preparación del semen congelado

- Descongelar pajuela en agua destilada (38.5°C) por 30 seg
- Colocar semen en medio SPERM-TALP por 10 min
- Centrifugar (2500G x 3min) – Lavar (repetir 3 veces)
- Analizar viabilidad y calcular concentración
- Diluir [2×10^6 x mL]

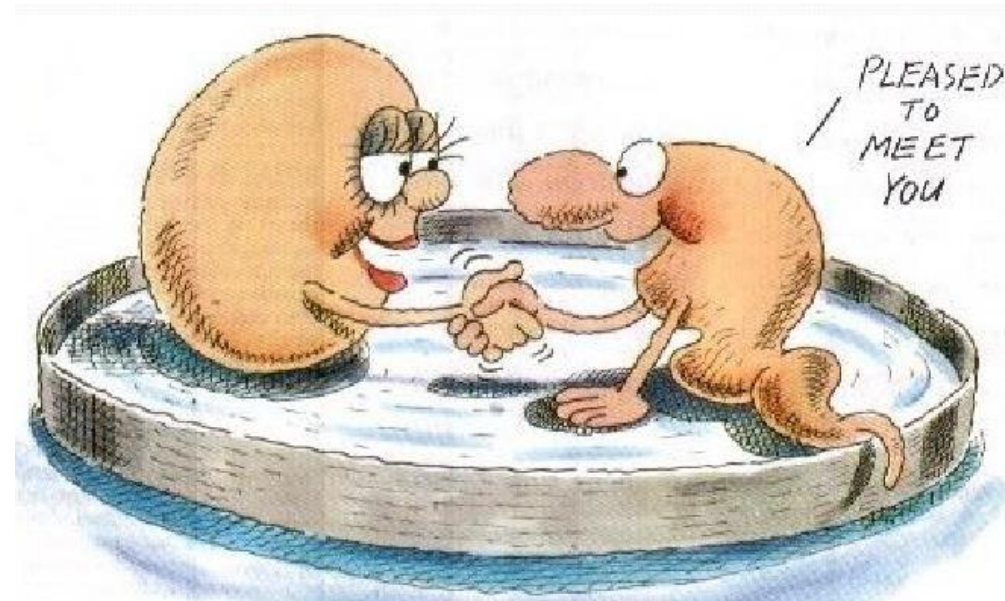


Fertilización in vitro

- Adicionar 250 uL de semen diluido en la gota con los ovocitos
- FECUNDAR!
- Incubar por 24hrs

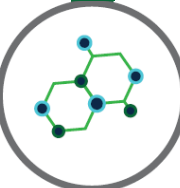
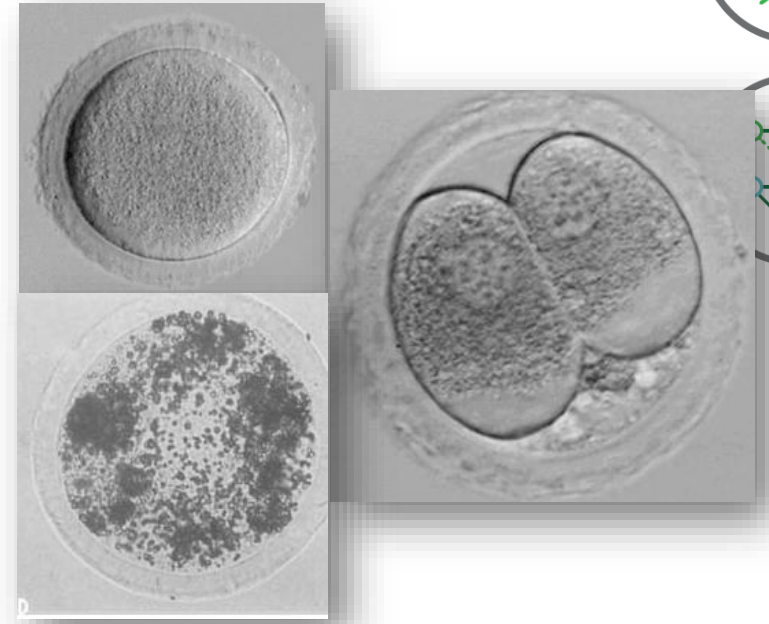
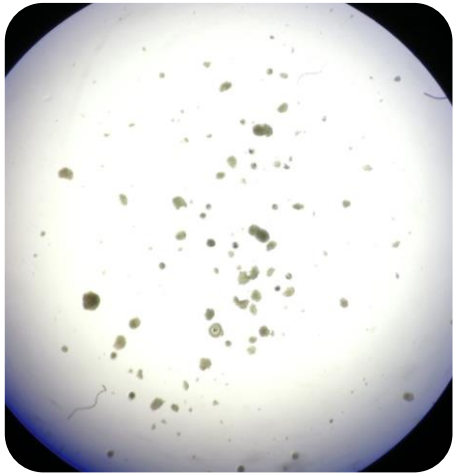


38.5°C, 5% CO₂



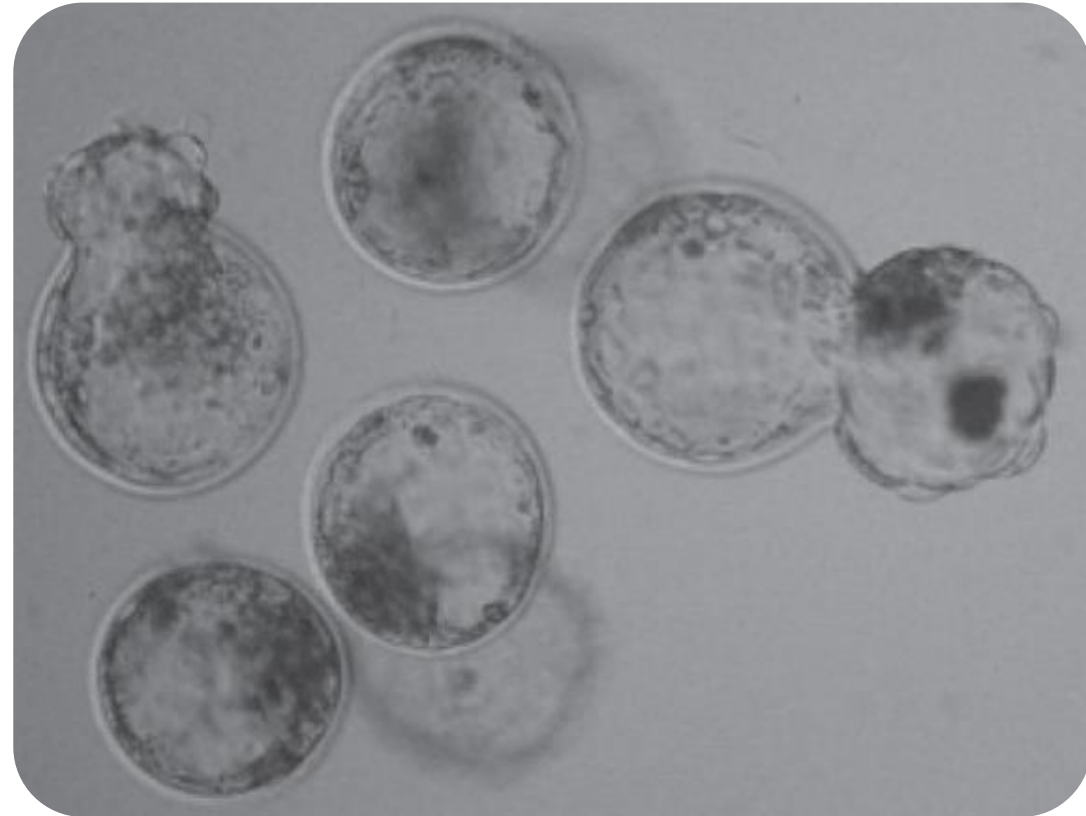
Producción in vitro de embriones

- Denudar los cigotos mediante vórtex por 3min
- Lavar los cigotos con PBS y medio SOF
- Transferir a placa con microgotas (25uL) – Máximo 25 cigotos por gota
- Luego de 24 hrs de incubación analizar divisiones
- Incubar hasta día 7/8



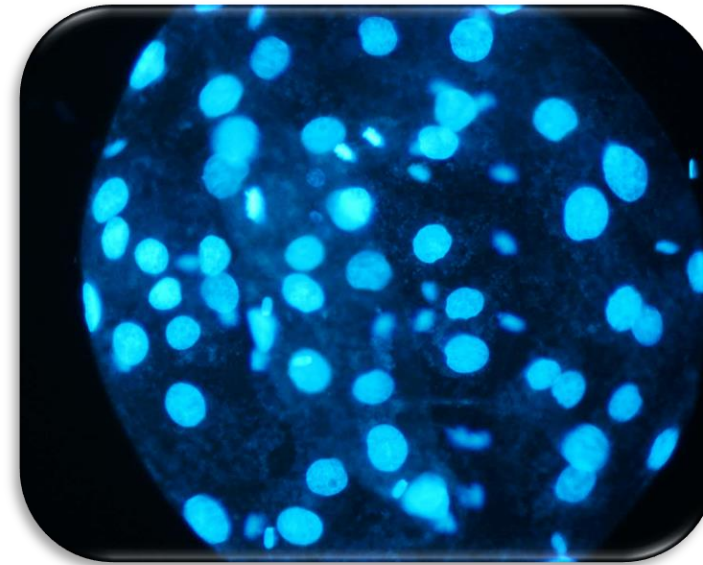
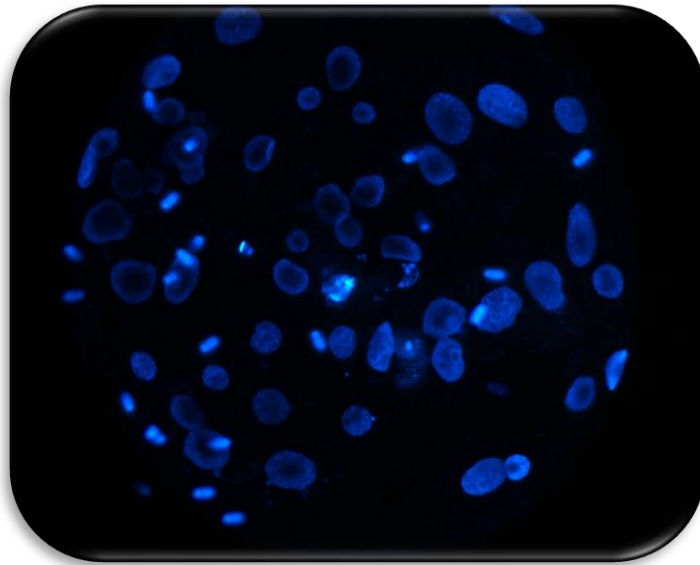
Control de cantidad de calidad embrionaria

- Al día 6, 7 y 8 analizar calidad embrionaria por número de:
 - Embriones
 - Mórulas
 - Blastocistos:
 - Temprano
 - Expandidos
 - Ecllosionados
- Análisis por fluorescencia



Control de calidad de calidad embrionaria

- Tinción con Hoechst 33342
- Conteo de blastómeras

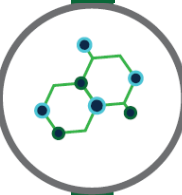


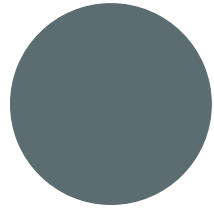
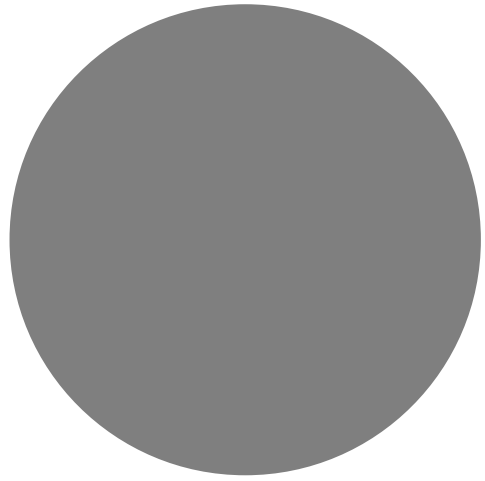
Resultados

Grupos	% Maduros	% Cigotos (2 células)	% Embriones (D6)	% Blastocistos (D7)
Control	90	50	45	40
Experimento 1	90	53	47	42
Experimento 2	90	60	52	55

7 réplicas de 30 – 50 ovarios

Datos sin estadística

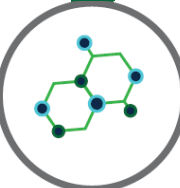
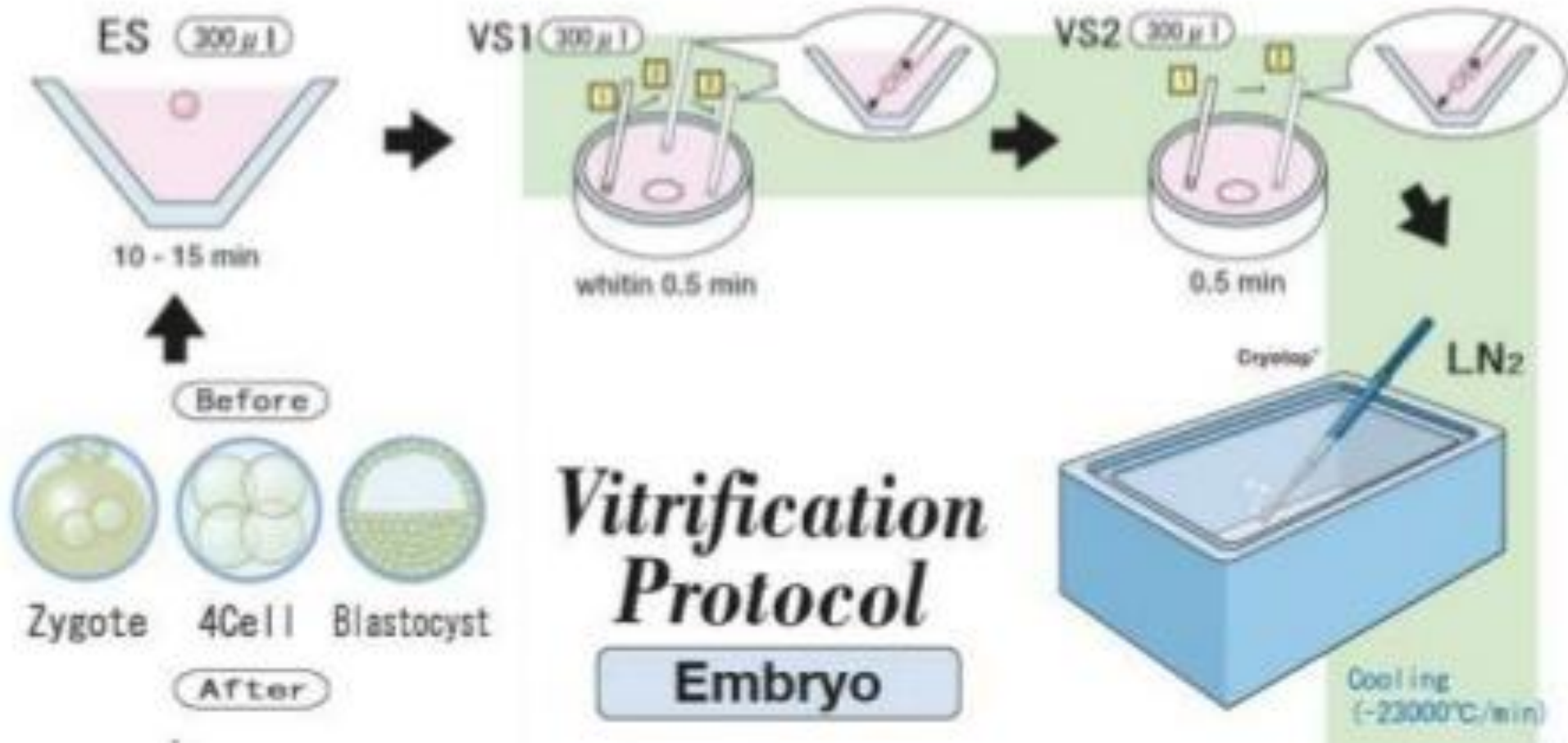


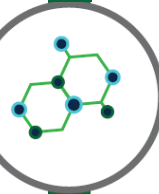


Criopreservación de embriones



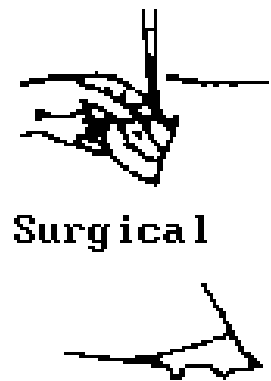
Vitrification Protocol Embryo







Transferencia embrionaria

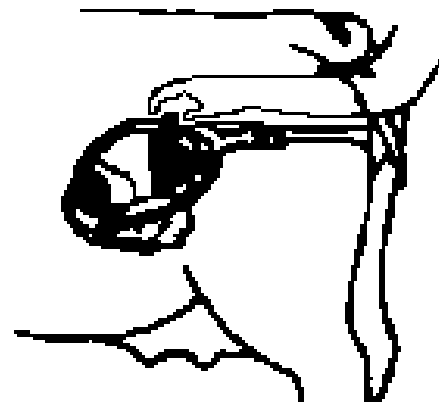


Surgical

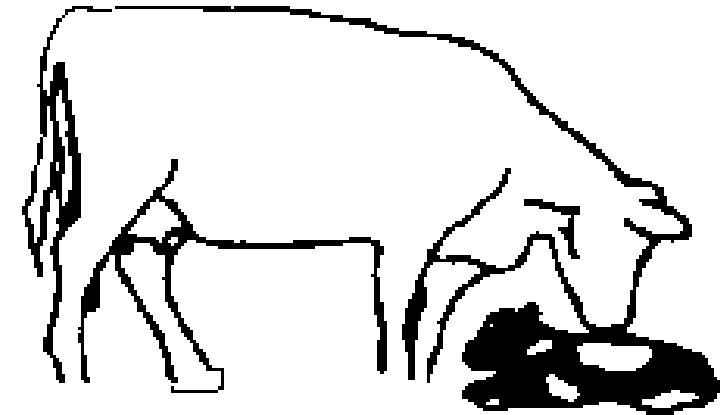


Non surgically

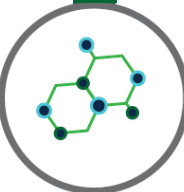
Transfer of embryos to recipients surgically or nonsurgically

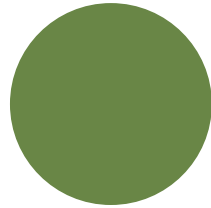
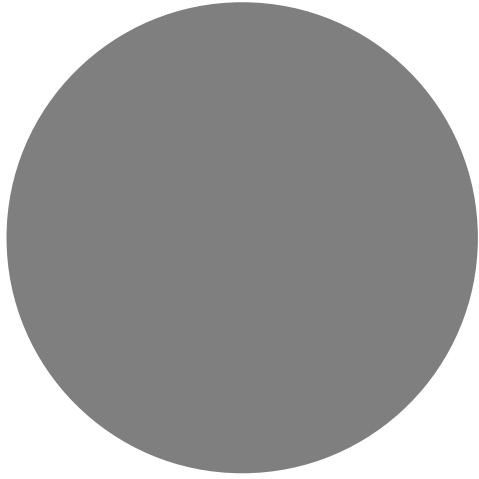


Pregnancy diagnosis by palpation through the rectal wall 1 to 3 months after embryo transfer



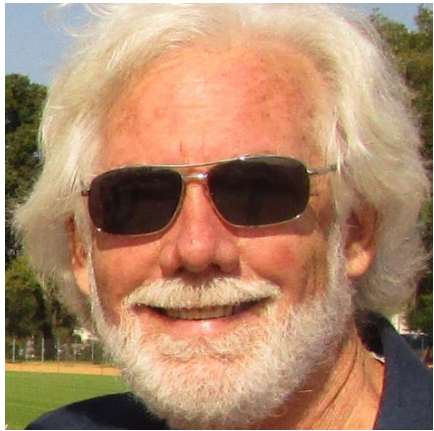
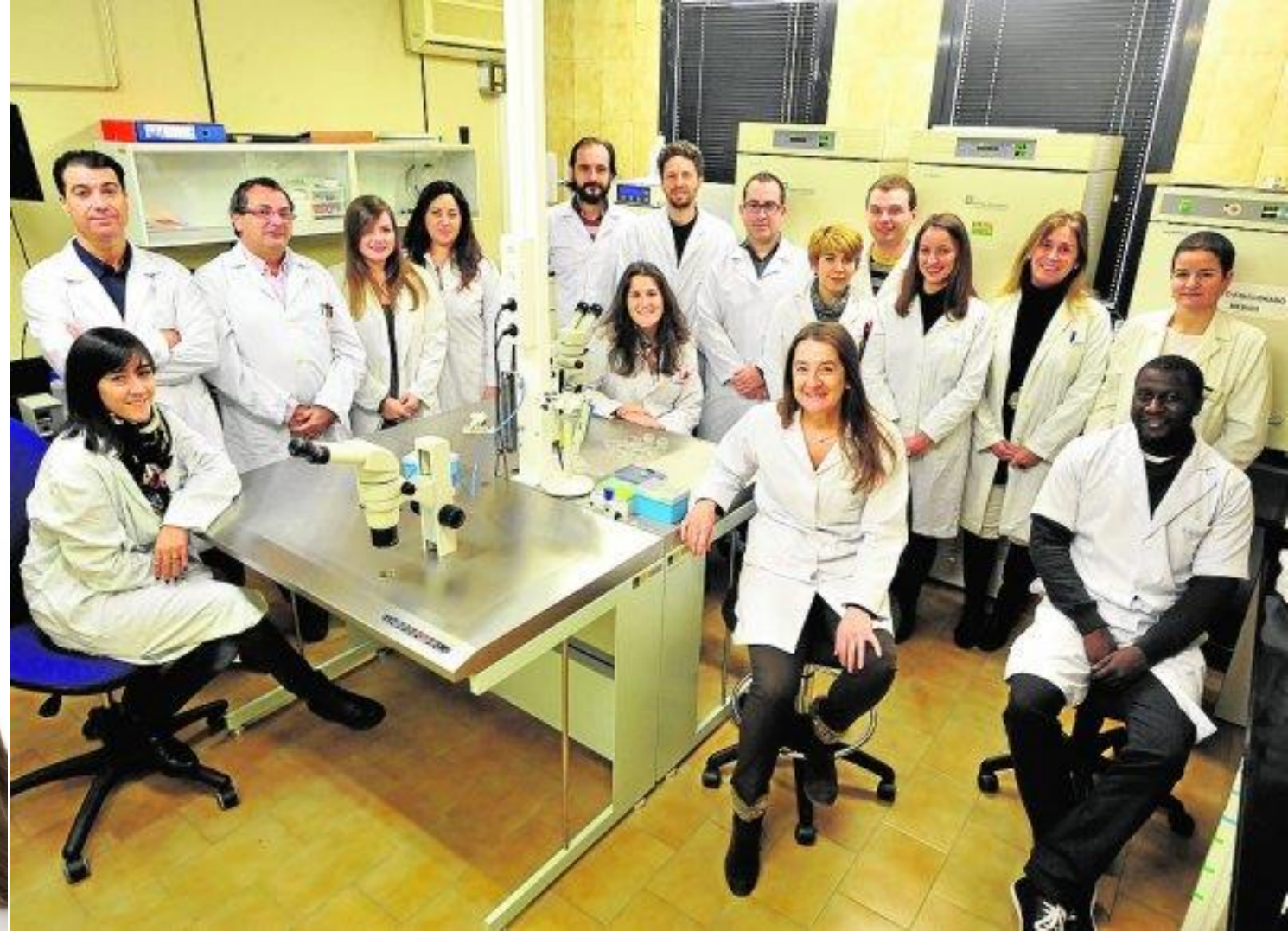
Birth (9 months after embryo transfer)





PROGRAMA PARAGUAYO PARA EL DESARROLLO DE LA CIENCIA Y TECNOLOGÍA

Experiencias del programa de
Vinculación de científicos y
tecnólogos



Lawrence P. (Larry) Reynolds, Ph.D
University Distinguished Professor,
Department of Animal Sciences, and
Director, Center for Nutrition
and Pregnancy
NORTH DAKOTA STATE UNIVERSITY



Francesca E. Duncan, PhD
Executive
Director, Center for Reproductive Science
Associate Research Professor
Department of Obstetrics and Gynecology
Feinberg School of Medicine
Northwestern University



PROGRAMA PARAGUAYO PARA EL DESARROLLO DE LA CIENCIA Y TECNOLOGÍA

UNIVERSIDAD DE
MURCIA



CENTRO PARA EL
DESARROLLO DE LA
INVESTIGACIÓN
CIENTÍFICA





Muchas gracias

Dra. Johanna Leiva
johileiva@gmail.com