FELINE IVF PROTOCOLS REVIEW

Neus Casamort Bonet – June 2016

Universitat Autònoma de Barcelona

FACULTAT DE VETERINÀRIA

AIMS

1. Review of scientific protocols about *in vitro* fecundation (IVF) in cats and creation of one in order to do the experimental part.
2. IVF of cat oocytes.

EXPERIMENTAL PROTOCOLS

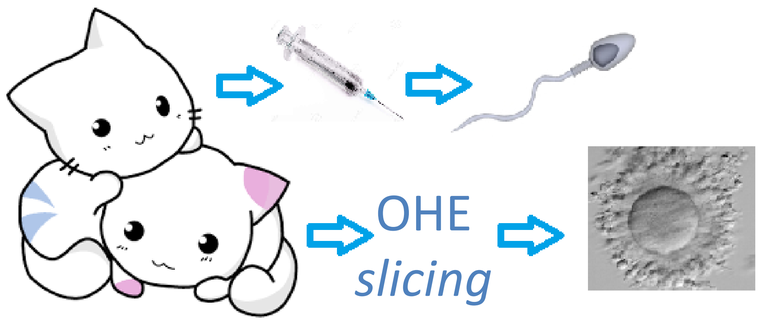

***Figure 1**: Gamete collection.*

GAMETE COLLECTION:

The experiments consulted used removed testis in order to obtain sperm. On the other hand, we prefered to use medetomidine.

CRYOPRESERVATION:

Used to preserve gametes (immature or mature), embryos or ovarian/testicular tissue. Needs cryoprotectants, which have cell toxicity.

Frequently used in the literature, but we had fresh sperm and oocytes.

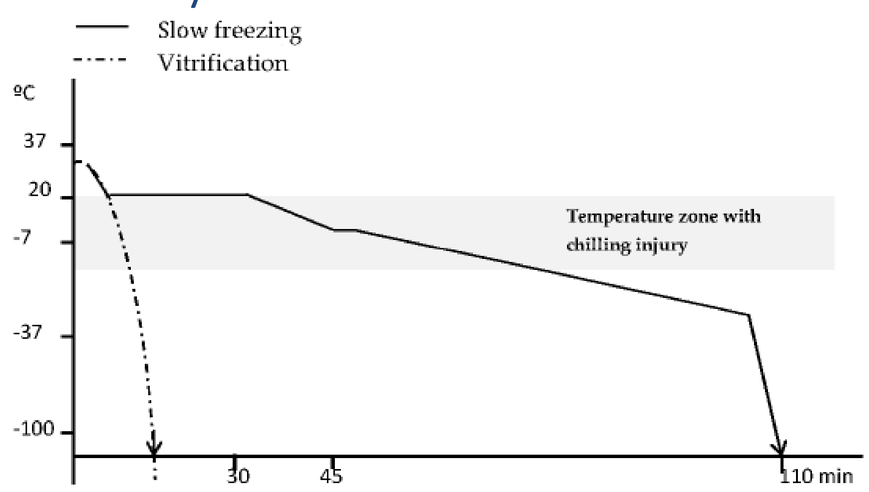

***Figure 2**: temperature decrease difference between slow freezing and vitrification. From http://www.interchopen.com/books/recent-advances-in-cryopreservation/the-maining-of-cryopreservation-for-in-vitro-fertilization-patients*

MADURATION:

Physiological changes in order to be fertilized. Maturation medium has to give all that cells require. We used TCM-199 with supplements at our laboratory.

FERTILIZATION:

Combine male and female gametes and culture them together in order to become embryos. Sperm has to be selected with swim-up and centrifugation. We used supplemented Tyrode medium.

REFERENCE	MADURATION MEDIUM	CULTURE CONDITIONS	RESULT
Alves et al. (2012)	Minimum essential medium (MEM)	38.5ºC 5% CO_2 6 days 15µl MEM changed every day for fresh.	52.6% follicular degeneration in oocytes matured without IGF. 45% follicular degeneration in oocytes matured with IGF. Oocytes recollected in luteal phase have worse results.
Alves, Kozel, Luvoni. (2012)	Krebs's Ringer Bicarbonate (mKRB)	38.5ºC 5% CO_2 24-48h	Viable: 93-94% Meiosis resumption: 65.8-67.5% *fresh oocytes data
Apparicio, Ruggeri, Luvoni. (2013)			8.7% germinal vesicles 82.6% meiosis resumption 8.7% degenerated *fresh oocytes data
Luciano et al. (2009)			20.3% germinal vesicles 1.7% intermediate 71.2% mature oocytes 6.8% degenerated
Luvoni, Pellizzari. (2000)			72.2% meiosis resumption 54.4% metaphase II
Luvoni et al. (2012)			85.9% meiosis resumption
Merlo et al. (2005)	SOFaaBSA	38ºC 5% CO_2 24h	67.3% meiosis resumption
Murakami et al. (2002)	25mM HEPES-buffered TCM-199	38ºC 5% CO_2 24h	It is an embryo development study. There are no specific results of intermediate stages like maturation, only the final ones.
Nagano et al. (2008)		39ºC 5% CO_2 0-48h	Maximum meiosis resumption is at 30h of culture (75.5%). Moreover, at that time also have maximum fertility (46.1%).

REFERENCE	FERTILIZATION MEDIUM	CULTURE CONDITIONS	RESULT
Comizzoli, Wild, Pukazhenthi (2006)	Hepes-Ham F10	$5x10^5$ sperm/mL 38.5ºC 5%CO_2 18h	90% fertility.
Merlo et al. (2005)	SOFaaBSA	$1.5x10^6$ sperm/mL 38.5ºC 5%CO_2 18h	Results are not specified.
Murakami et al. (2002)	Brackett-Oliphant (BO)	$2x10^6$ sperm/mL 38ºC 5%CO_2 12h	It is an embryo development study. There are no specific results of intermediate stages like fertilization, only the final ones.
Nagano et al. (2008)	Brackett-Oliphant (BO)	$1.5x10^6$ sperm/mL 39ºC 5%CO_2 18h	24.8% fertility.

RESULTS

Our maturation protocol had negative results.

This kind of experimental design have complex steps. The literature, certainly defines culture mediums, but it does not specify details. Sadly, the time required to improve the protocol and obtain some results is more than the time we had, probably months. We decided to expand the bibliographic and theory part instead.

CONCLUSIONS

There is not much information and experiments about feline reproduction than production animals. It seems that each laboratory started with a different protocol and there is no standard one.

The final goal to acomplish is the reproduction of endangered wild felines. Therefore, I recomend an experimental comparation of the more used protocols in order to create an standard.

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