Research Topics and Seminar
(501)
Dr. Hassan Al-Haddad

In vitro embryo production in camel (Camelus dromedarius) from in vitro matured oocytes fertilized with epididymal spermatozoa stored at 4 ◦C

Name: Zahraa Almomen

Date: 3 May 2021

ELSEVIER

Animal Reproduction Science 111 (2009) 69–79

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

In vitro embryo production in camel (*Camelus dromedarius*) from in vitro matured oocytes fertilized with epididymal spermatozoa stored at 4 °C

N.A. Wani *

Central Veterinary Research Laboratory, Post Box 597, Dubai, United Arab Emirates

Received 23 August 2007; received in revised form 6 February 2008; accepted 6 February 2008
Available online 13 February 2008

Abstract

Experiments were conducted to study the effect of storing epididymal spermatozoa, in tris–tes- and tris–lactose egg yolk extenders, on their fertilizing ability and subsequent in vitro embryo development. Ovaries and testes were collected from a local slaughterhouse in normal saline solution (NSS) at 37 °C and on ice (0–1 °C), respectively. Cumulus oocyte complexes (COCs) aspirated from the follicles were randomly distributed to 4-well culture plates (20–25 COCs/well) containing 500 μL of maturation medium and cultured at 38.5 °C in an atmosphere of 5% CO_2 in air for 36 h. Spermatozoa were collected from the cauda epididymides in syringes containing 2–3 mL of either tris–tes- or tris–lactose egg yolk extender. They were cooled down slowly and stored at refrigeration (4 °C) temperature. The spermatozoa were evaluated for motility and used for IVF of IVM oocytes on the day of collection and after 2, 4, 6 and 8 days of storage. On the day of IVF, spermatozoa were prepared by the swim up technique and both spermatozoa and oocytes were co-incubated at 38.5 °C in a humidified atmosphere of 5% CO_2 in air for 15–16 h. Presumptive zygotes were either fixed and stained with Hoechst 33342 for evaluation of fertilization or were cultured in 500 μL of the culture medium at 38.5 °C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 in air. There was no significant difference ($P > 0.05$) in the proportion of oocytes fertilized with spermatozoa stored in either of the two extenders for up to 8 days. The proportion of oocytes that cleaved (43–60%) and those that developed to blastocysts (14–21%) did not show any difference ($P > 0.05$) either, when spermatozoa from different days of storage were used. First cleavage was observed as early as 16 h after IVF, early blastocysts had developed by day 4, expanded blastocysts after day 5 and hatching of blastocysts started after day 6 of culture.

* Tel.: +971 4 3375165; fax: +971 4 3368638.
E-mail addresses: nwani@cvrl.ae, drnawani@hotmail.com.

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2008.02.005

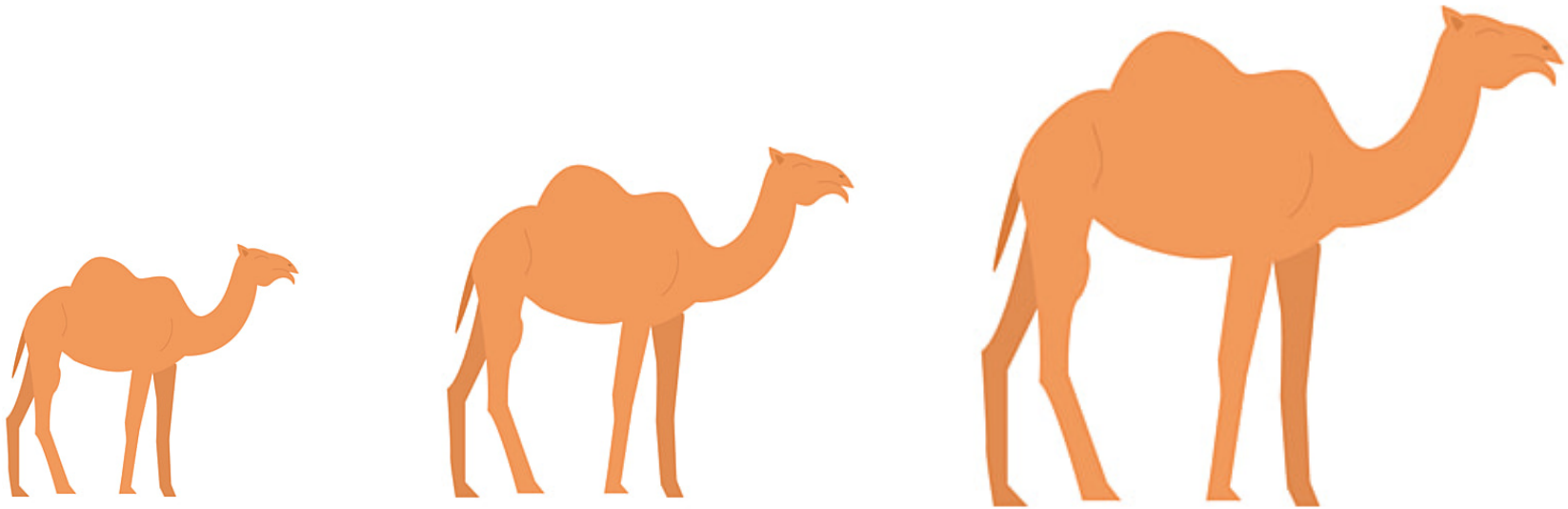
Outline

- Introduction (general information about camel and Breeding)
- Objective
- Method
- Result
- Discussion
- Conclusion

Introduction

Camel

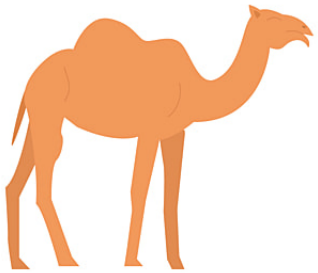
- Scientific name of the camel is *Camelus*.
- Camel can live for 40 years.



EMBRYO

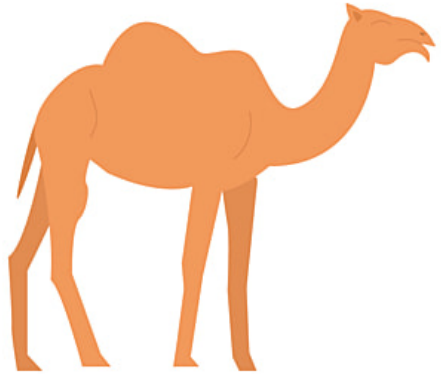
Sexual maturity

- Male: at 5 years.
- Female: at 3 to 4 years



IMMATURE

Years



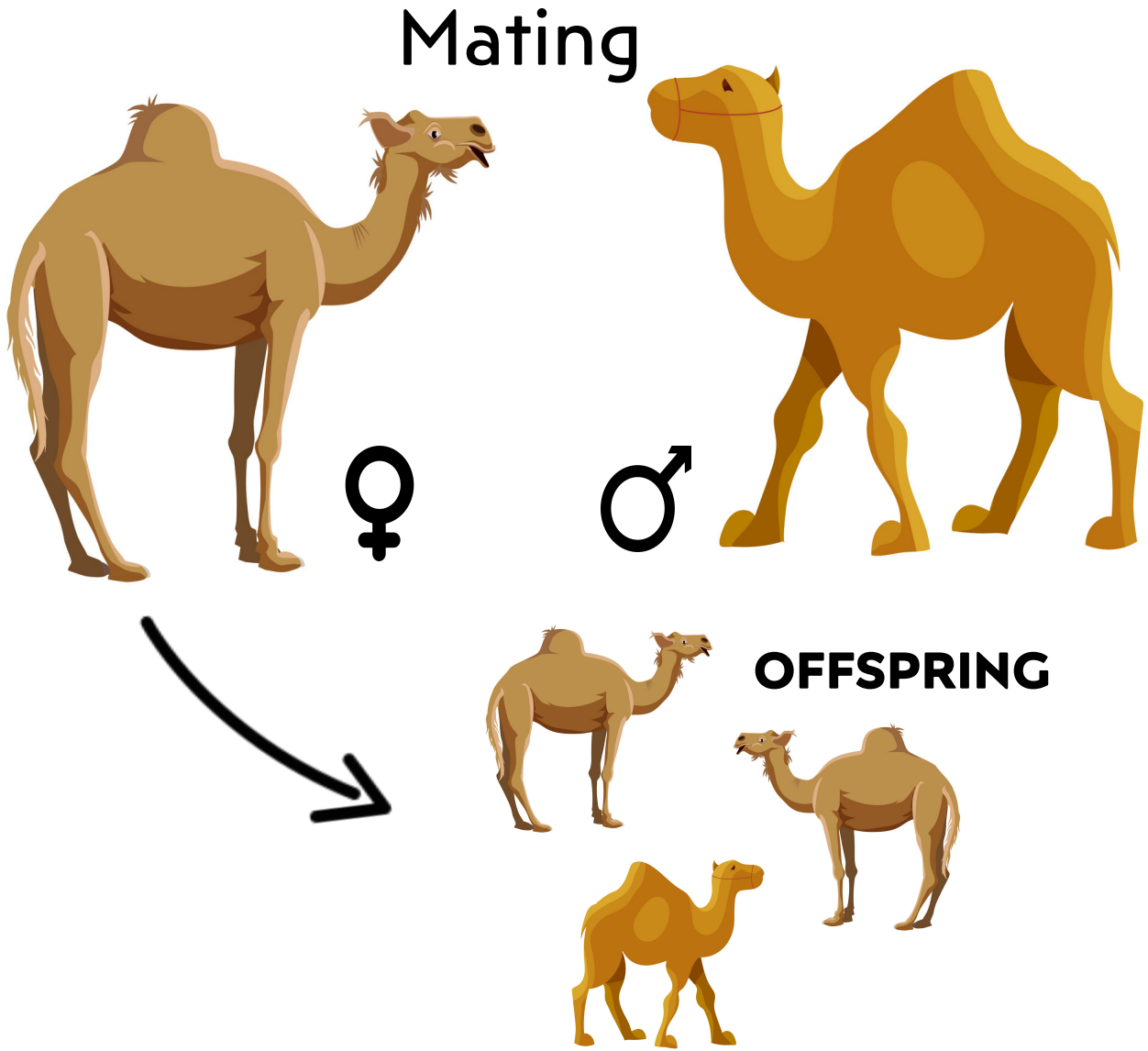
MATURE

Breeding season

Sudan	betweenMarch and August
Egypt	December to April
India	November to February

Reproduction

- It happens when male camel mates female camel.
- This is the natural way.



Camel pregnancy

- Pregnancy is characterized by the presence of a large and well developed CL (CORPUS LUTEUM) which is maintained throughout pregnancy.
- The gestation length takes from 315 to 440 days.
- Birth weight of dromedary calves varies from 19kg to 52 kg

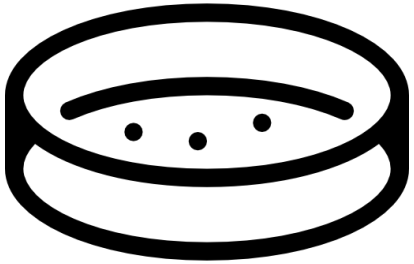
In vitro fertilization

- It is the artificial way in which it happened in the lab.

Lab

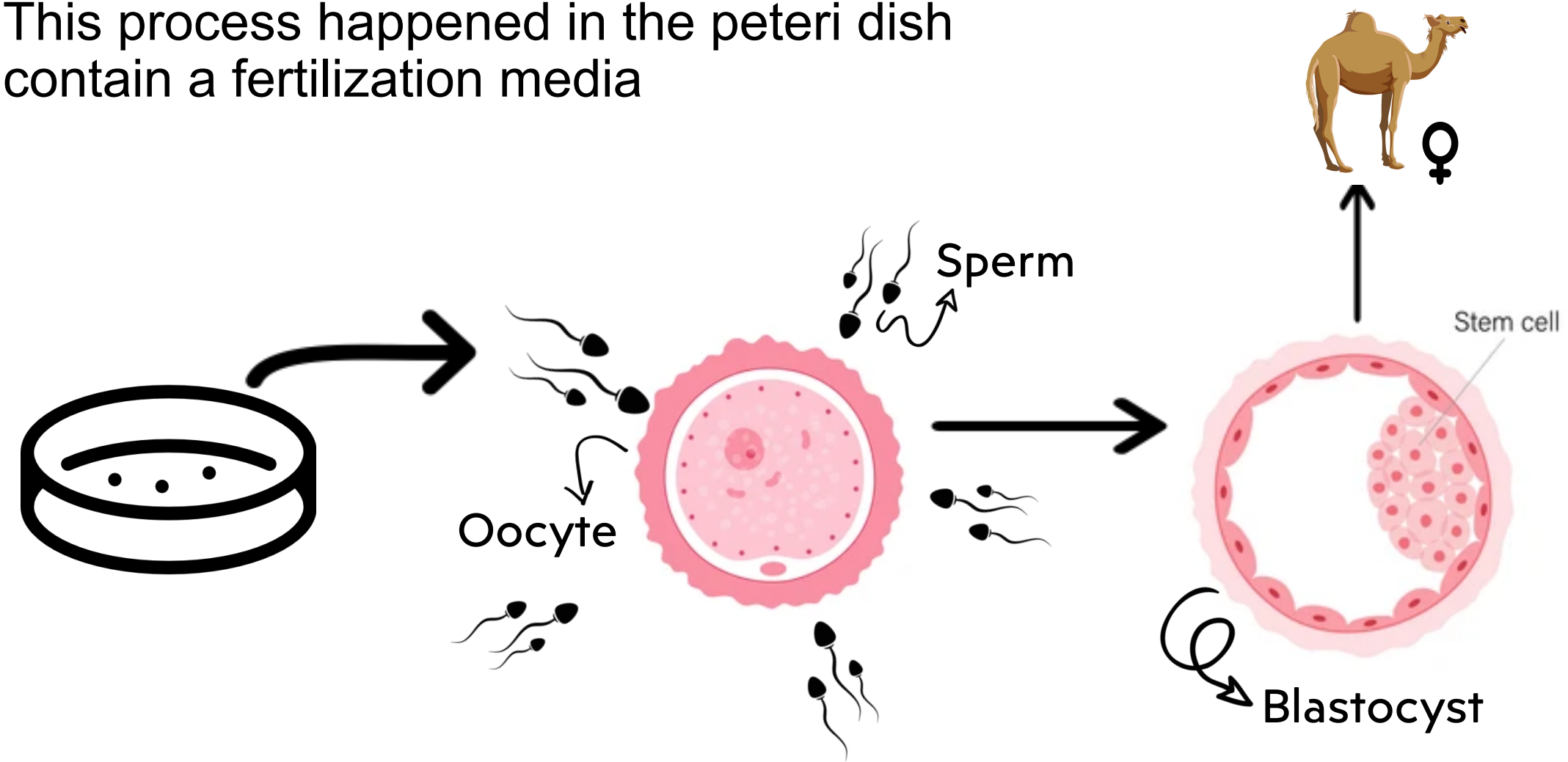
Embryologist

Petri dish



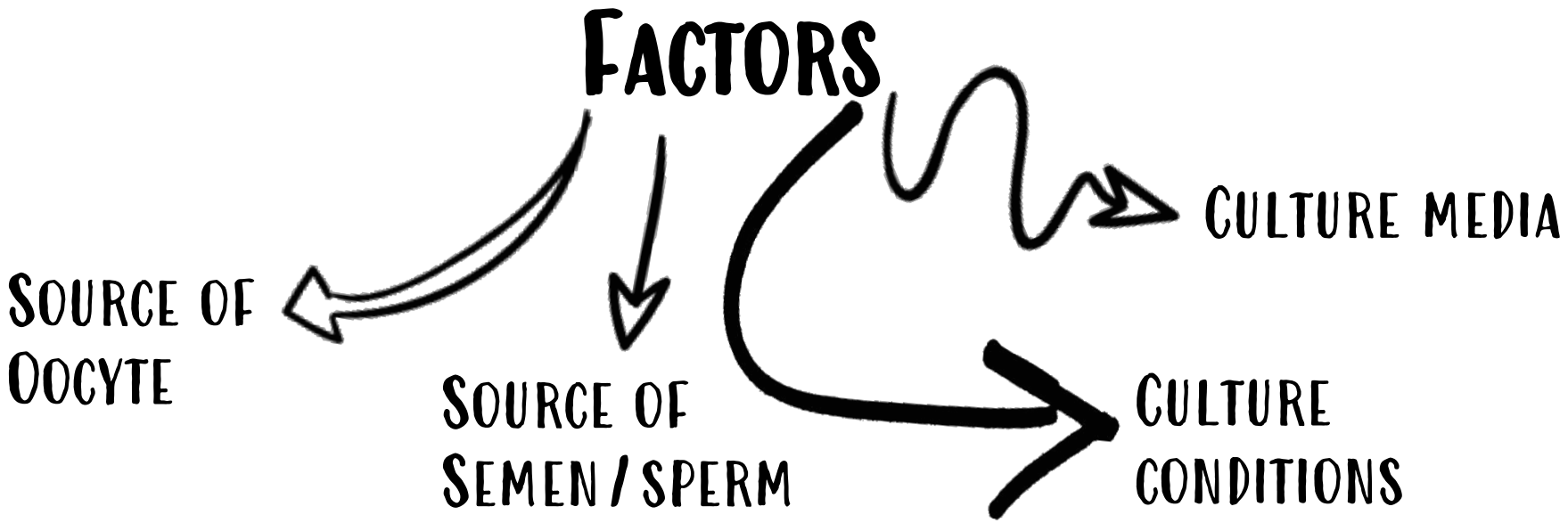
In vitro fertilization

- This process happened in the peteri dish contain a fertilization media



In vitro fertilization

- There are many factors affecting the process



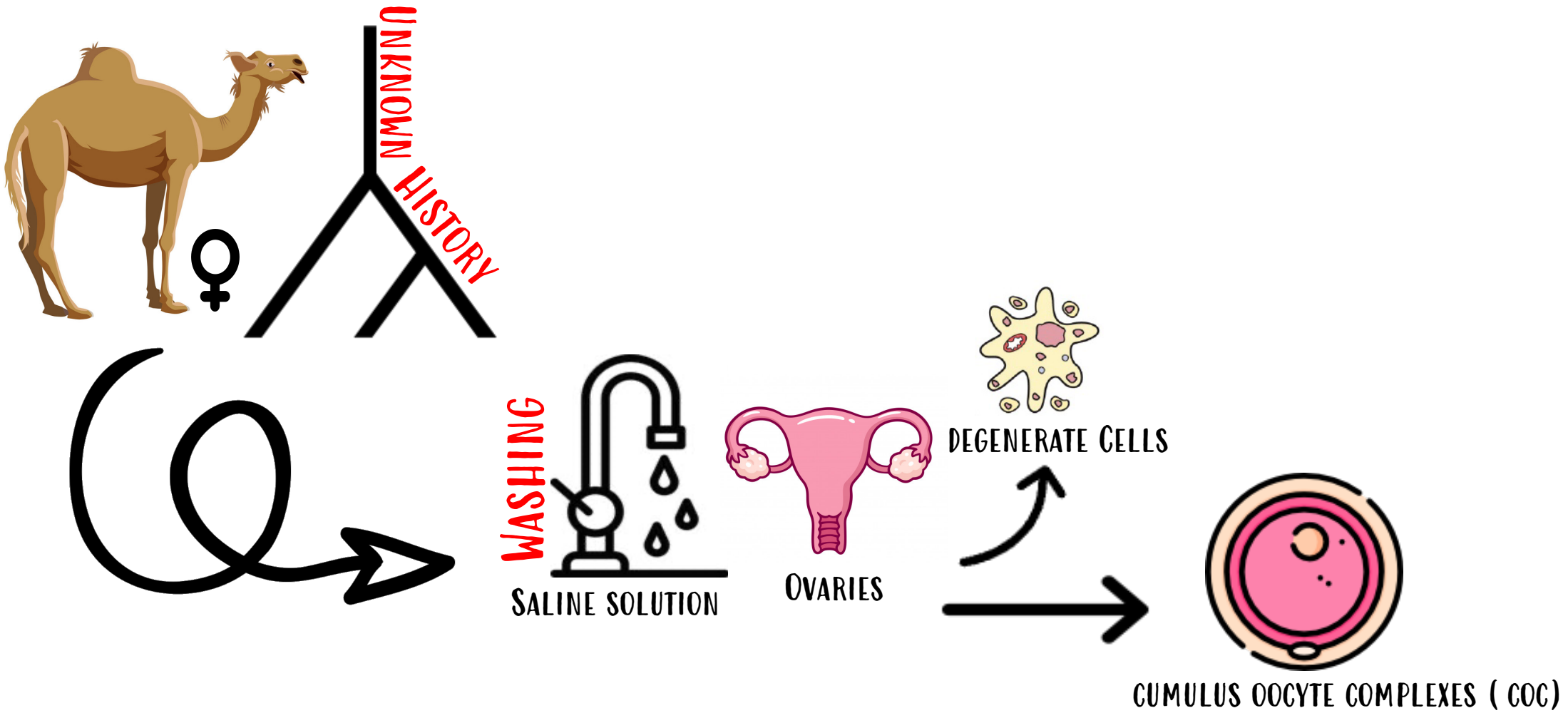
Objective

Objective

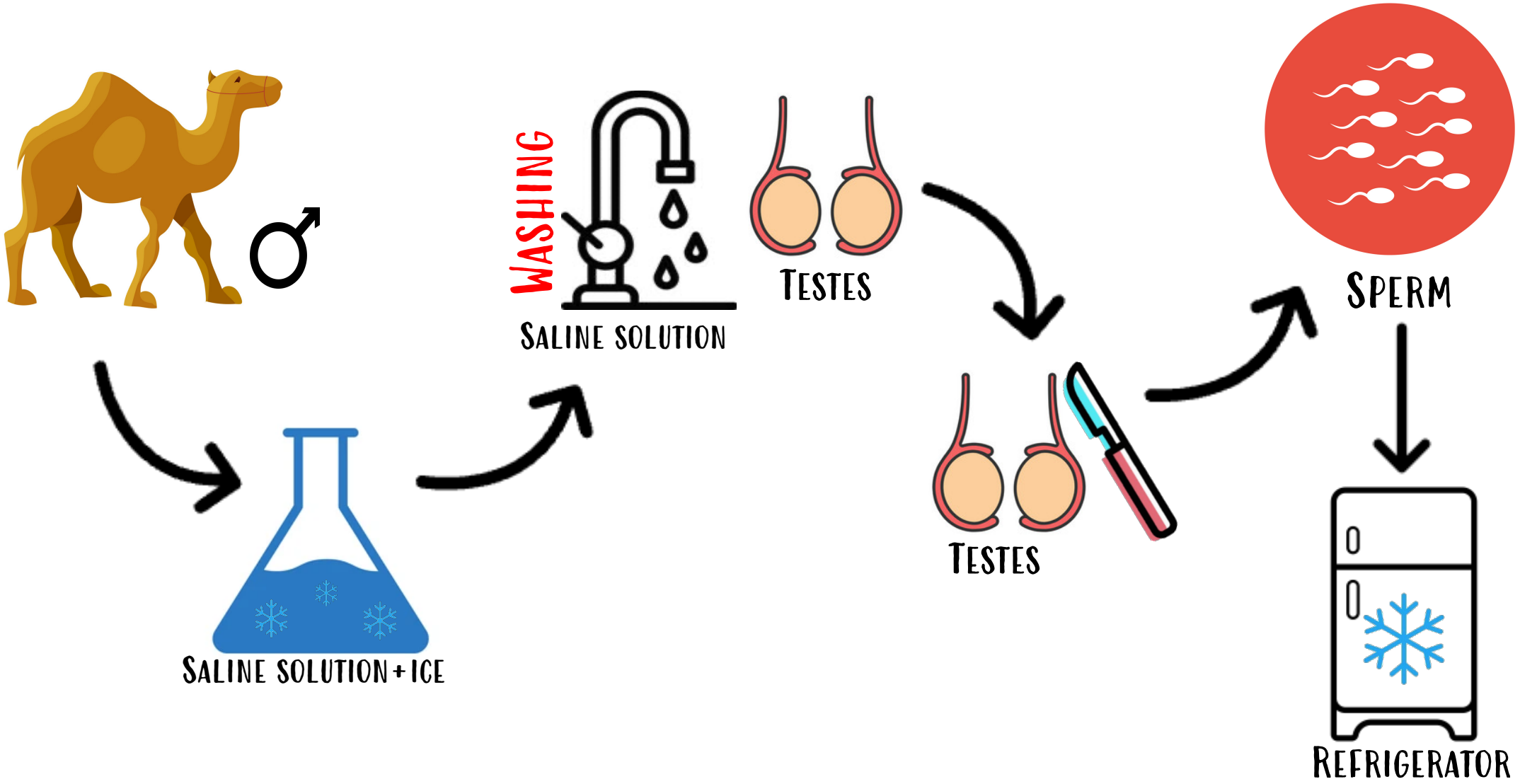
- To determine the effect of storing epididymal spermatozoa on fertilizing ability of the sperm.

Material and methods

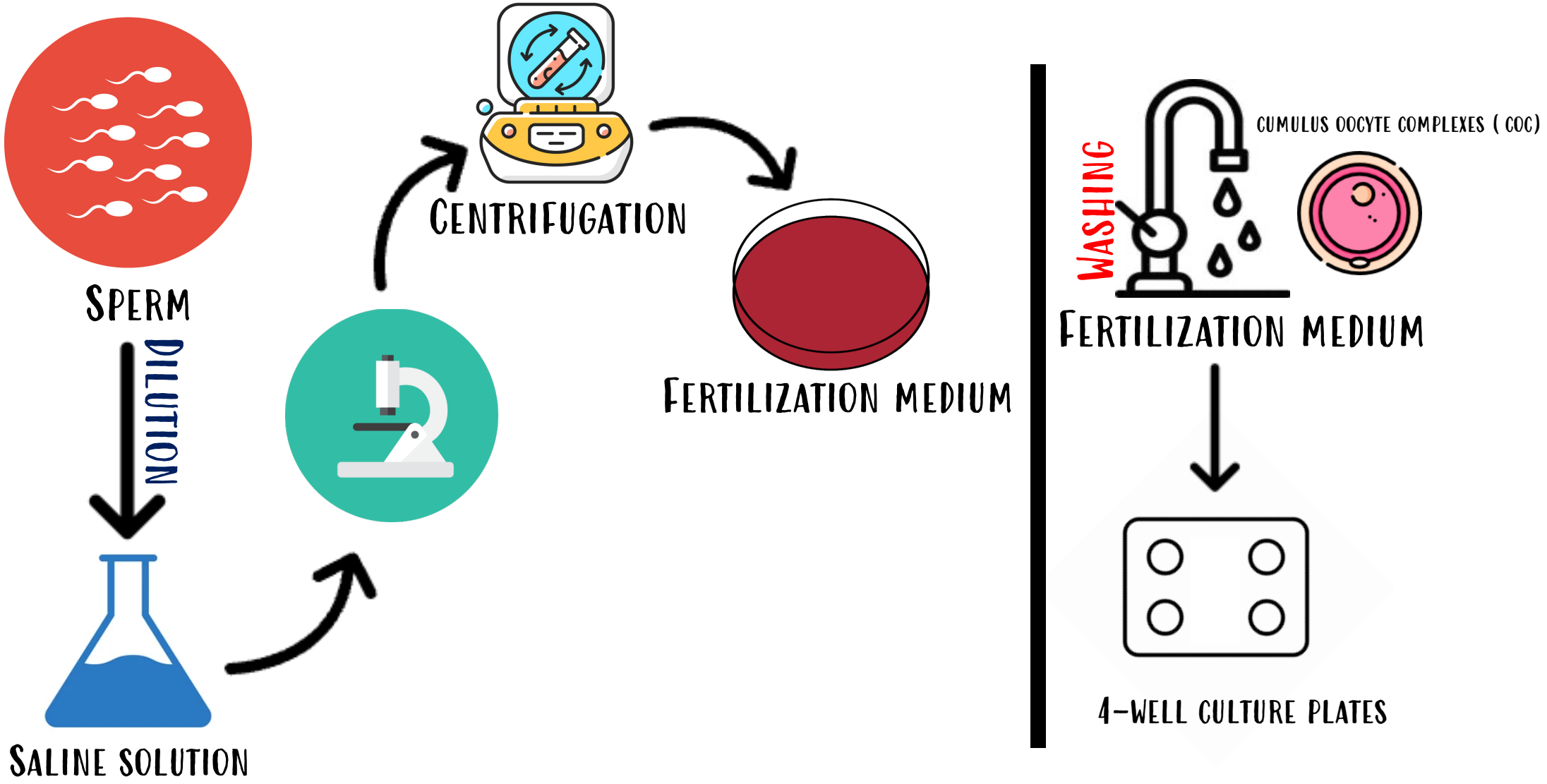
Collection of COC



Collection and storage of semen

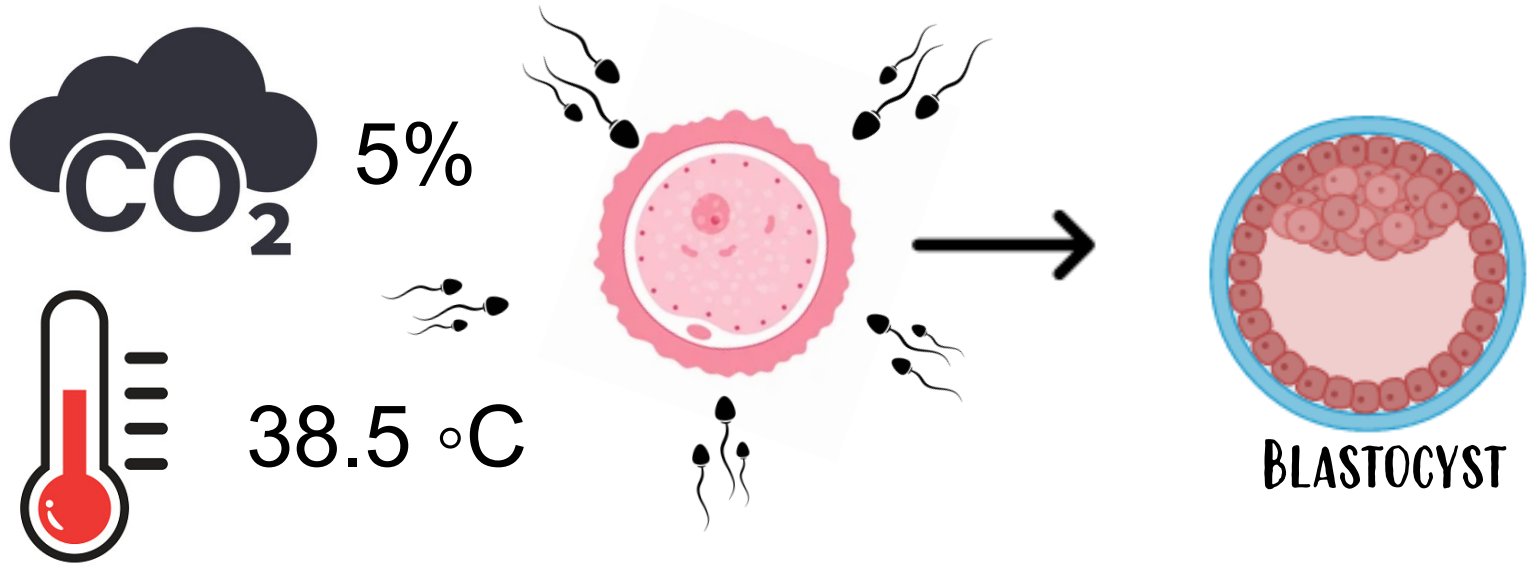


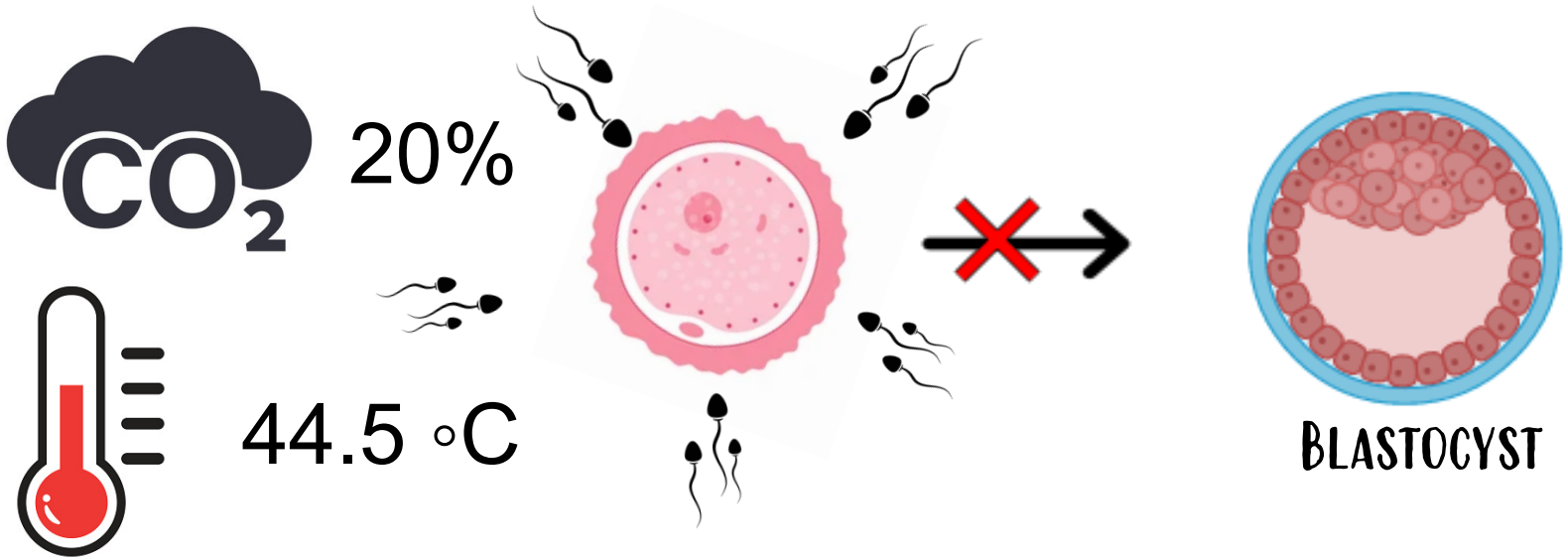
In vitro fertilization



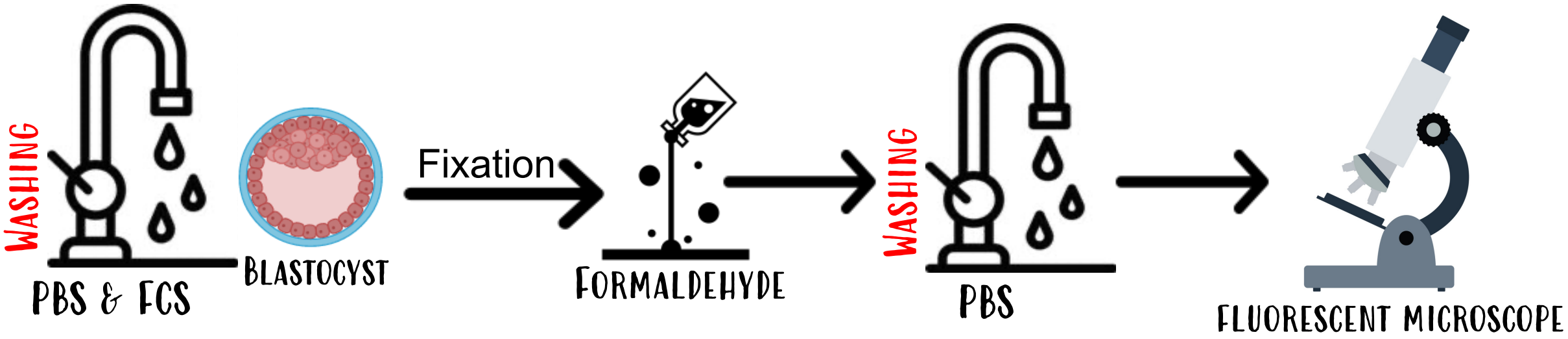
Conditions:

- There are conditions that enhance the fertilization process.





Evaluation of fertilized oocyte



Statistical Analysis

- Some statistical tests were done:

I. T-test

II. Chi- square test

Result

In vitro fertilization

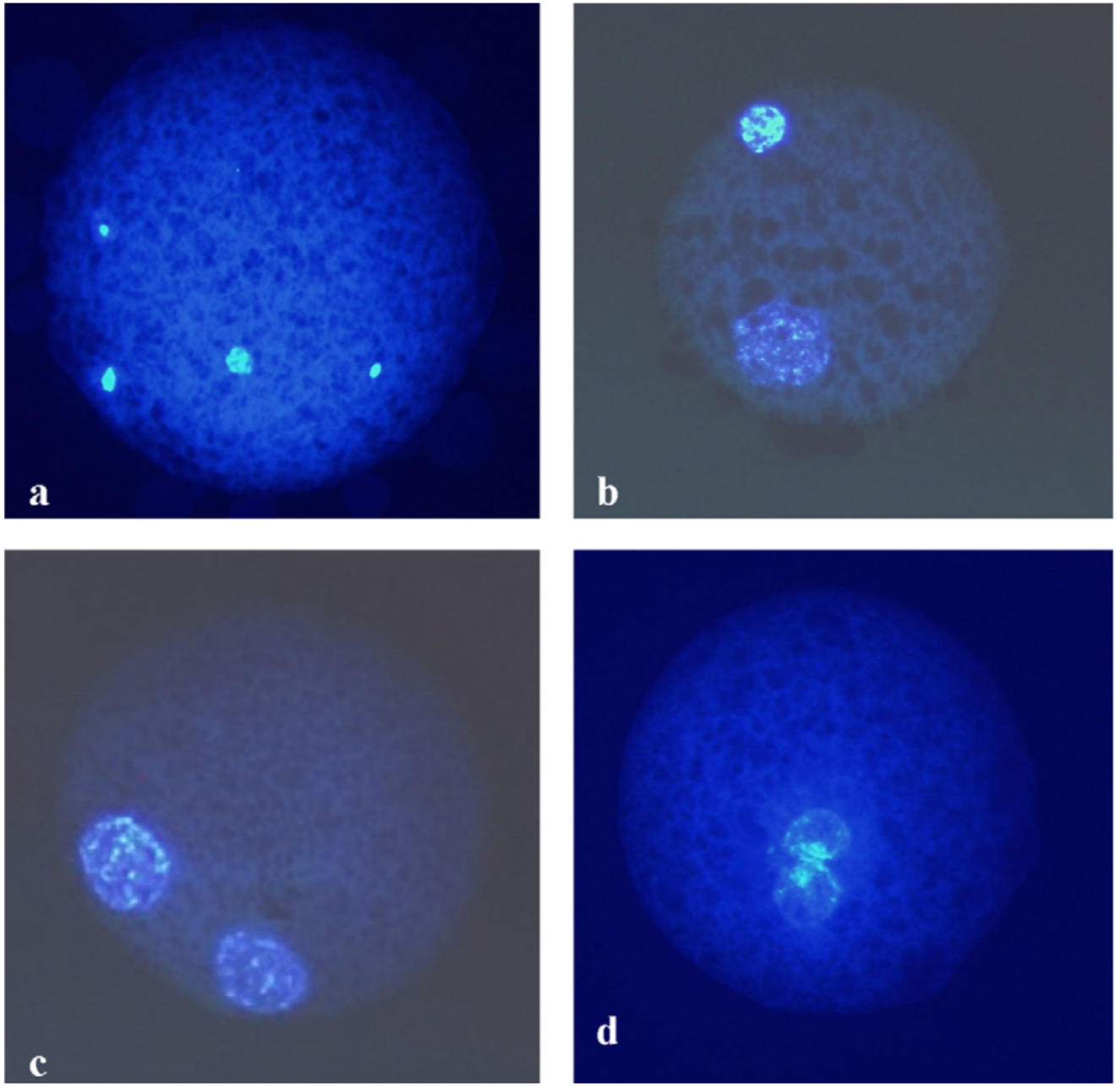
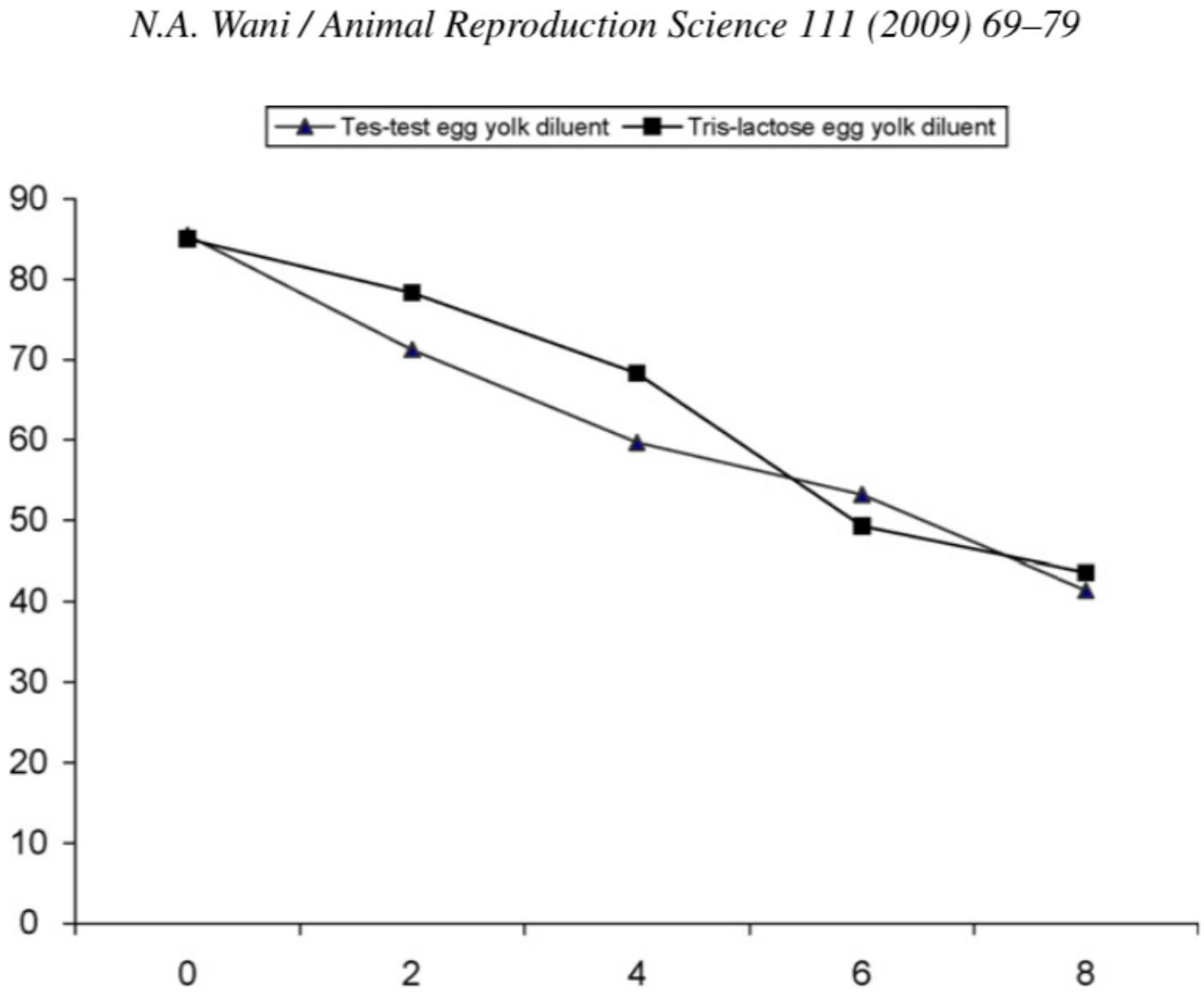


Fig. 1. In vitro fertilized oocytes: (a) with a sperm in ooplasm and visible first and second polar bodies; (b) with a female pronucleus and condensation of sperm chromatin; (c) male and female pronuclei and (d) a syngamous male and female pronuclei.

Sperm motality

- There was slow loss of spermatozoa motility.
- There was no difference in motality between Two types of sperm



Fertilization rate

Table 1
Fertilization rates of the in vitro matured oocytes with the epididymal spermatozoa stored in tris–tes- and tris–lactose egg yolk extenders at 4 °C up to 8 days

Day of semen storage	Extender used	Total oocytes (%)			
		Inseminated	Fertilized normal	Polyspermic	Total
0	Tris–tes	102	58 (56.9)	3 (2.9)	61 (59.8)
	Tris–lactose	92	59 (64.1)	3 (3.2)	62 (67.4)
2	Tris–tes	96	58 (60.4)	4 (4.2)	62 (64.6)
	Tris–lactose	90	60 (66.7)	3 (3.3)	63 (70.0)
4	Tris–tes	93	50 (53.8)	7 (7.5)	57 (61.3)
	Tris–lactose	92	57 (61.9)	5 (5.4)	63 (68.5)
6	Tris–tes	95	49 (51.6)	5 (5.3)	54 (56.8)
	Tris–lactose	90	55 (61.1)	3 (3.3)	58 (64.4)
8	Tris–tes	89	51 (57.3)	7 (7.9)	57 (64.0)
	Tris–lactose	97	59 (60.8)	2 (3.3)	61 (62.9)

Development of matured oocyte

Table 2
Development of in vitro matured oocytes fertilized with epididymal spermatozoa stored in tris–tes- and tris–lactose egg yolk extenders at 4 °C up to 8 days

Day of semen storage	Extender for semen storage	Total oocytes inseminated	Cleaved (%)	Blastocyst			Hatched blastocysts (%)[c]
				No	(%)[a]	(%)[b]	
0	Tris–tes	119	56 (47)	11	9	20	4 (36)
	Tris–lactose	115	61 (53)	13	11	21	5 (38)
2	Tris–tes	120	66 (55)	14	12	21	5 (36)
	Tris–lactose	117	70 (60)	15	13	21	8 (40)
4	Tris–tes	124	65 (52)	10	8	15	3 (30)
	Tris–lactose	121	70 (58)	14	12	20	5 (36)
6	Tris–tes	113	52 (46)	8	7	15	2 (25)
	Tris–lactose	109	60 (55)	11	10	18	3 (27)
8	Tris–tes	123	57 (46)	8	6	14	2 (25)
	Tris–lactose	129	56 (43)	10	8	18	4 (33)

[a] Percent from total oocytes.
[b] Percent from cleaved embryos.
[c] Percent from blastocysts.

Blastocyst development

- First cleavage was seen after 16 h from IVF.
- Early blastocysts had developed by day 4.
- Expanded blastocysts after day 5 and hatching of blastocysts started after day 6 of culture.

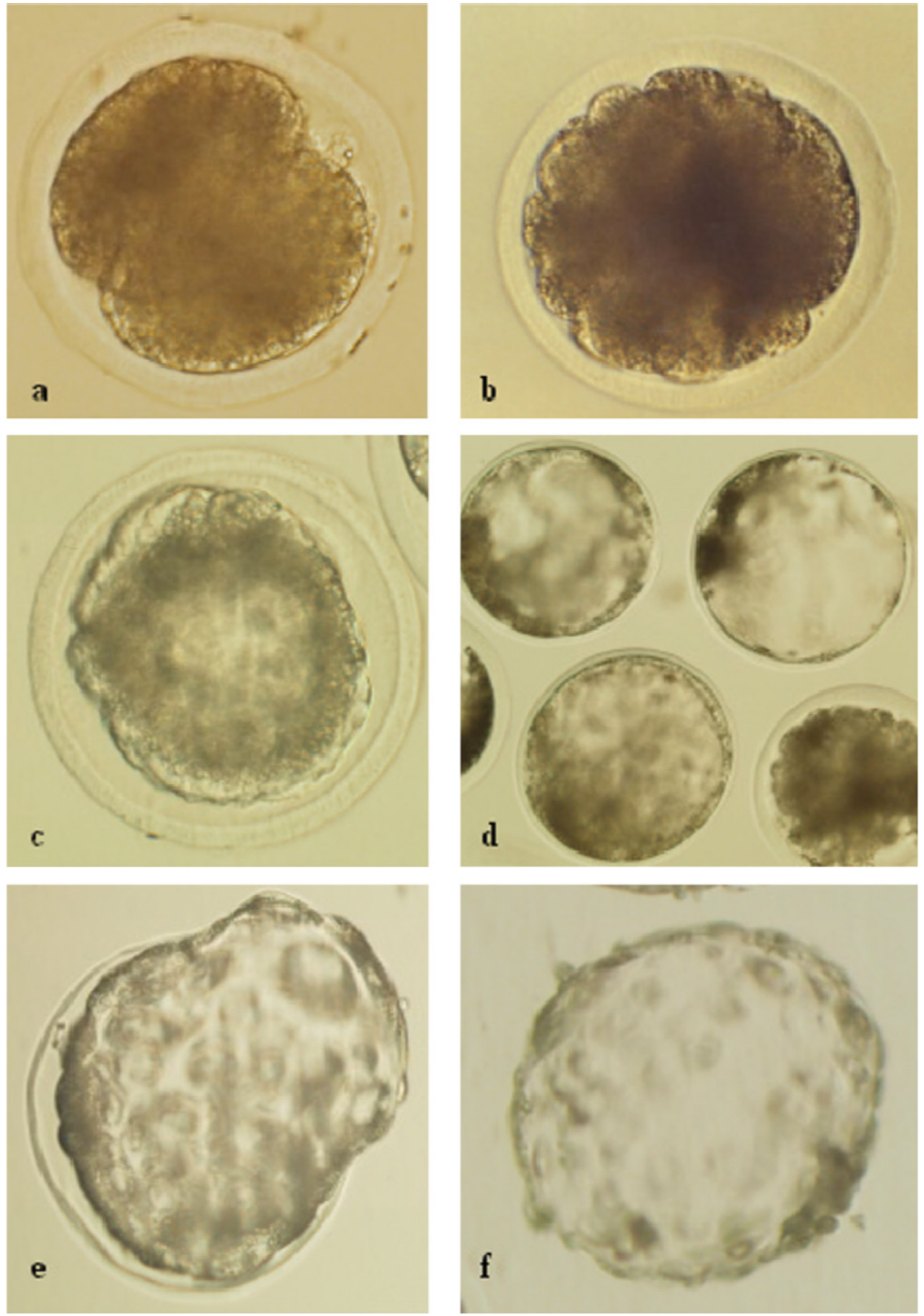


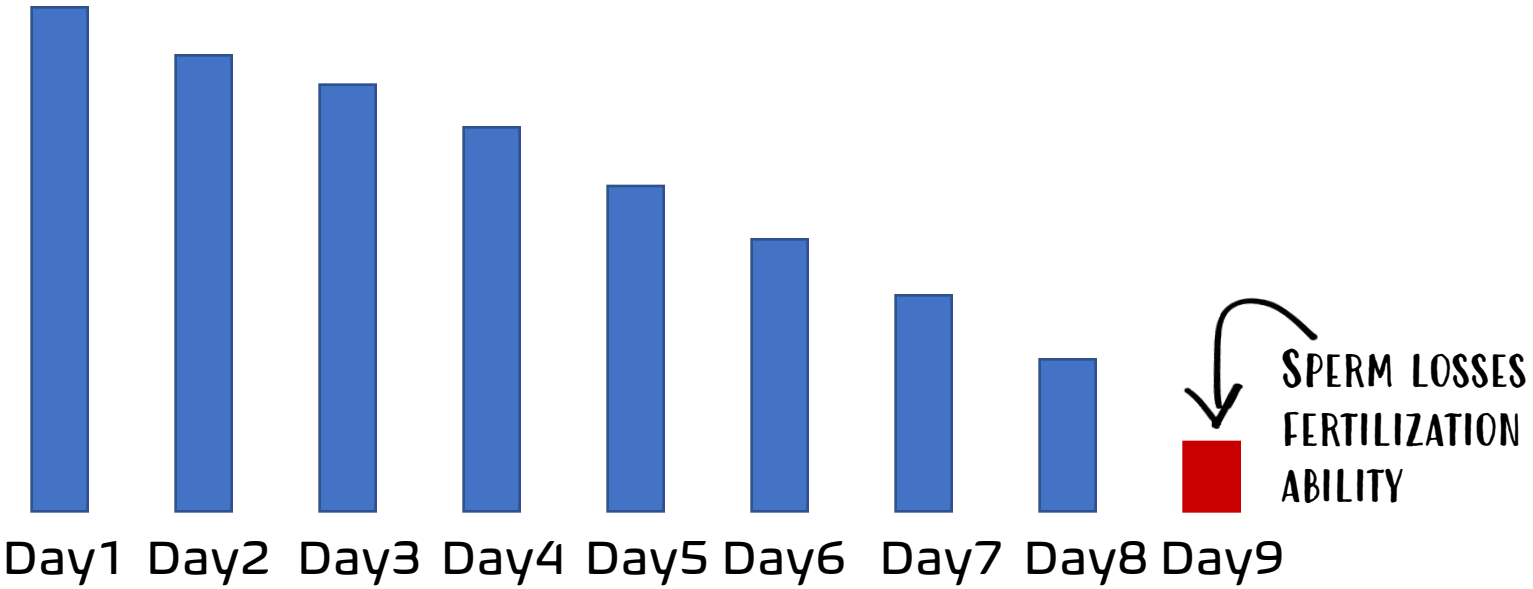
Fig. 3. In vitro embryo production in dromedary camel: (a) a 2-cell embryo; (b) a morula; (c) an early blastocyst; (d) expanded blastocysts; (e) a hatching blastocyst and (f) a hatched blastocyst.

Discussion

Fertilization ability

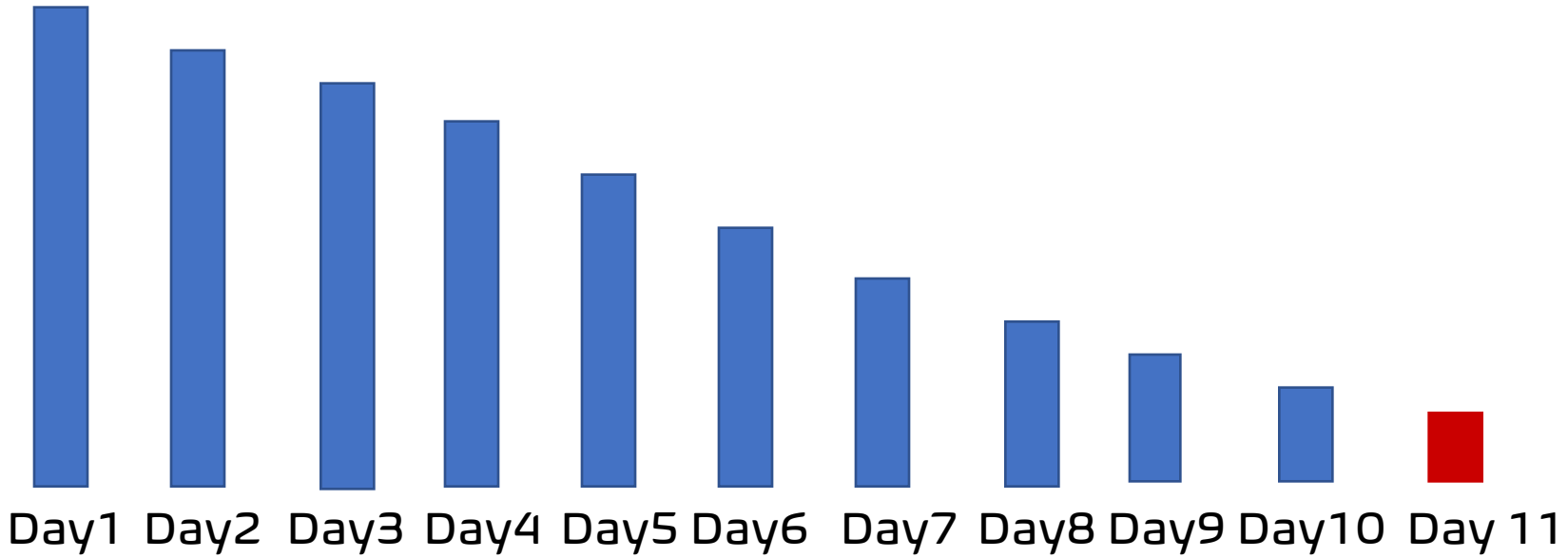
- Epididymal spermatozoa was able to fertilize mature oocytes in vitro after storage at 4 ◦C in tris–lactose and tris–tes extenders for at least 8 days.

Camel



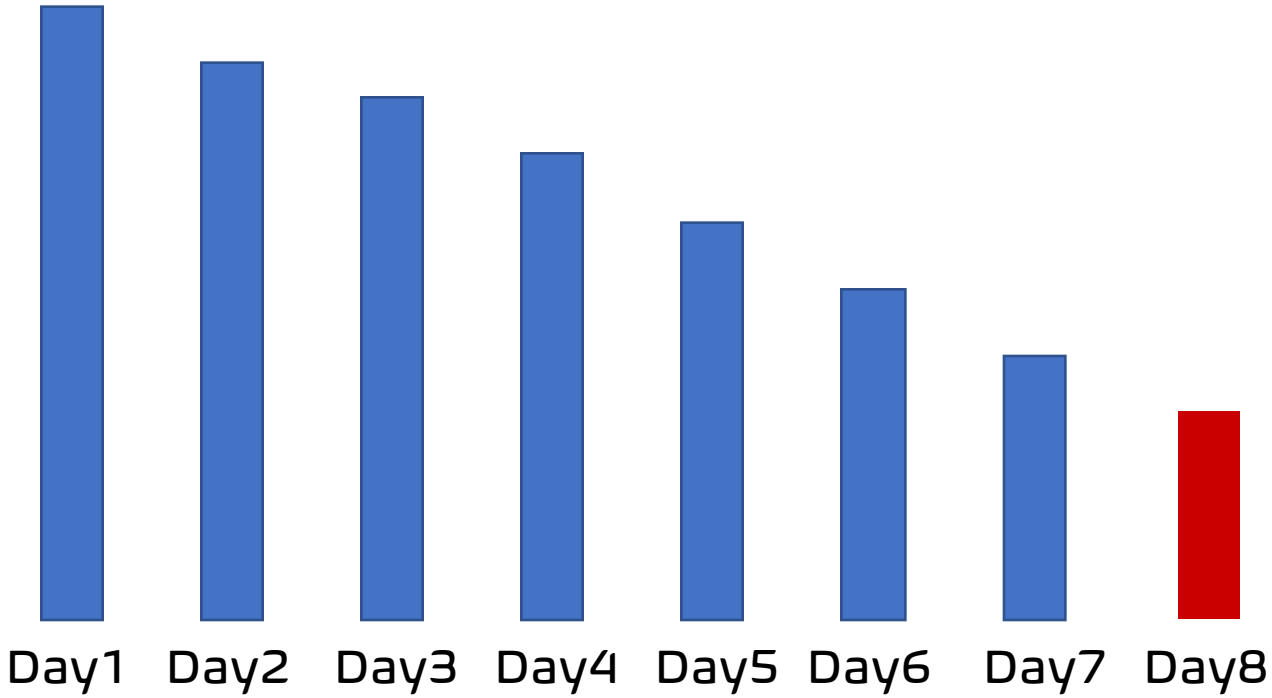
Fertilization ability

Bull



Fertilization ability

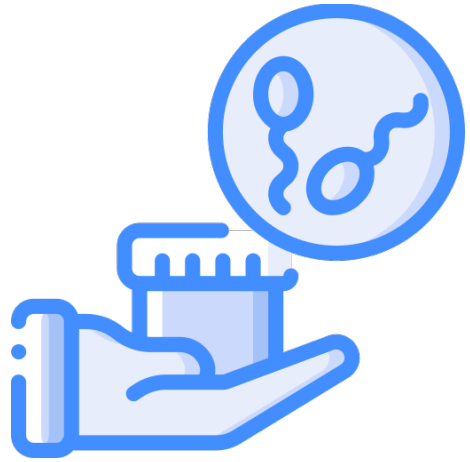
Cat



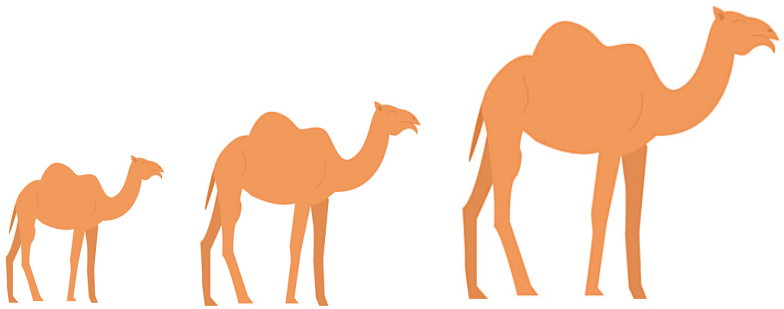
Fertilization rate

- The fertilization rate of epididymal spermatozoa may differ due to:

Site of sperm collection



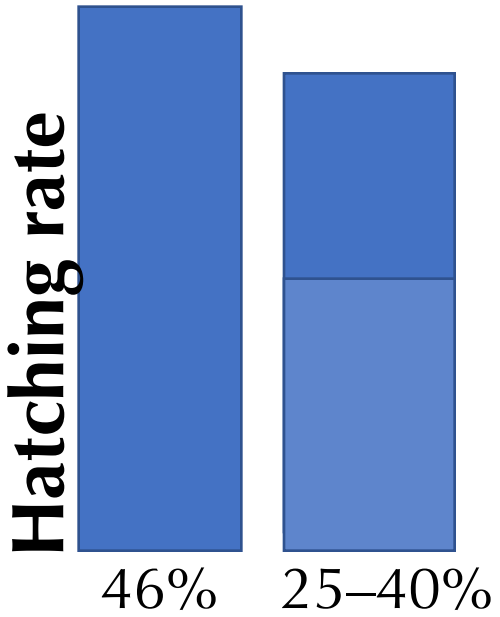
methods of semen preparation



age of the animals

Hatching rate

- Hatching rate was slightly lower than the study published before.
- The reasons for this variability might be the supplements in embryo culture medium.



(Khatir and Anouassi, 2006)

Embryo development

- The chronology of embryo development in dromedary camels is faster than in other species.
- In cattle the blastocyst is seen after 6–7 days of culture and hatches after day 7 while in llamas the blastocysts hatch on day 7 after IVF.

16 h post IVF	first cleavage was observed
Day 6	first hatched blastocysts

Conclusion

Conclusion

- The dromedary epididymal spermatozoa survive in storage for at least 8 days in tris–lactose and tris–tes egg yolk diluents at 4◦C.
- These spermatozoa maintain their fertilizing ability and may be suitable for use in IVF.

Reference:

- Wani, N., 2009. In vitro embryo production in camel (Camelus dromedarius) from in vitro matured oocytes fertilized with epididymal spermatozoa stored at 4°C. *Animal Reproduction Science*, 111(1), pp.69-79.
- Eiwishy, A., 1987. Reproduction in the female dromedary (Camelus dromedarius): A review. *Animal Reproduction Science*, 15(3-4), pp.273-297.