In-vitro maturation of camel oocytes using different media and sera

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Why?

Economic role of dromedary camel
- Milk
- Meat

Therapeutic effect (Knoess, 1984; Abdalla et al., 2015).
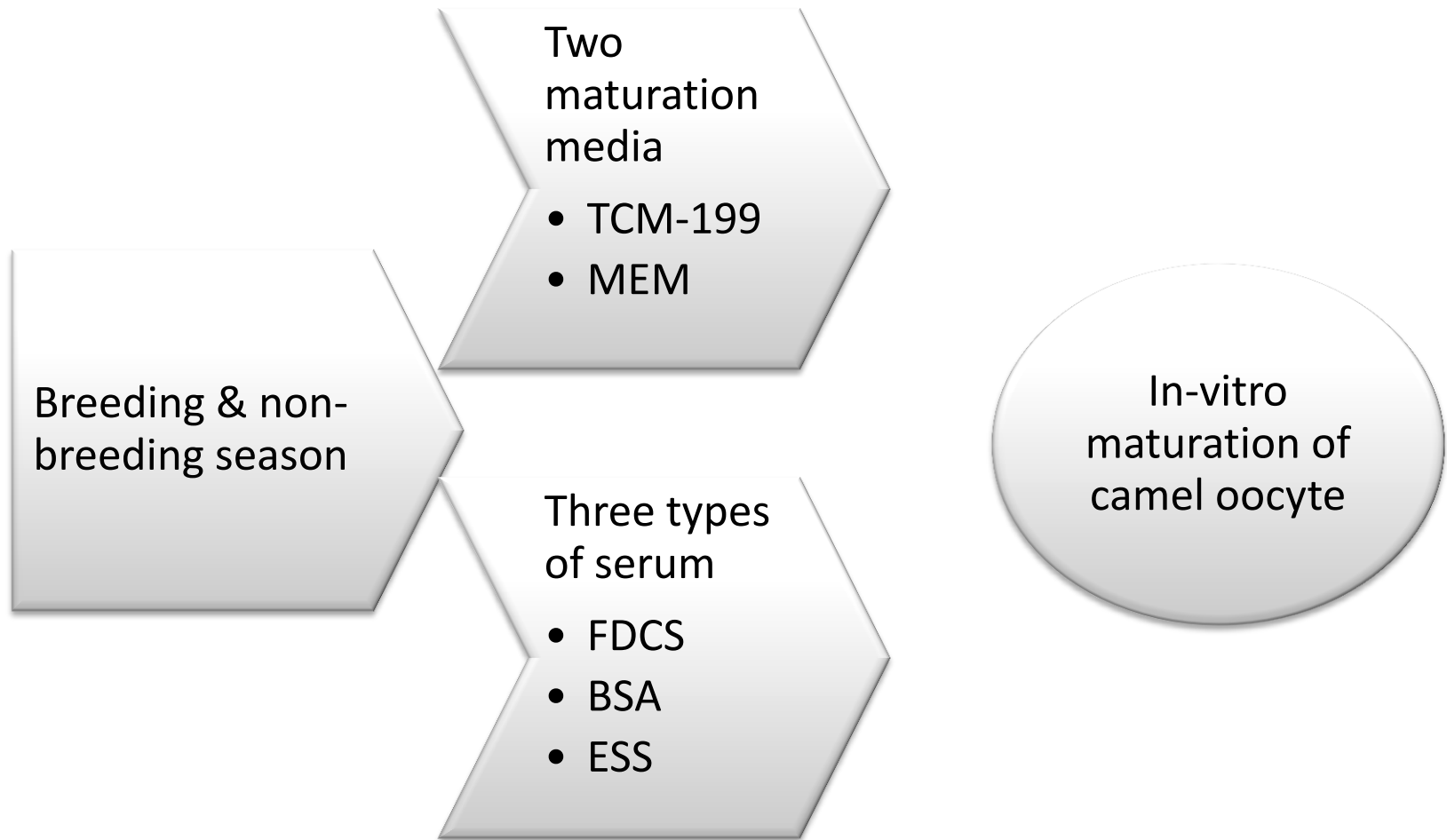
Seasonality of reproduction
- Male (Allam et al., 2013).
- Female (Shalash, 1980; Kandil et al., 2014).
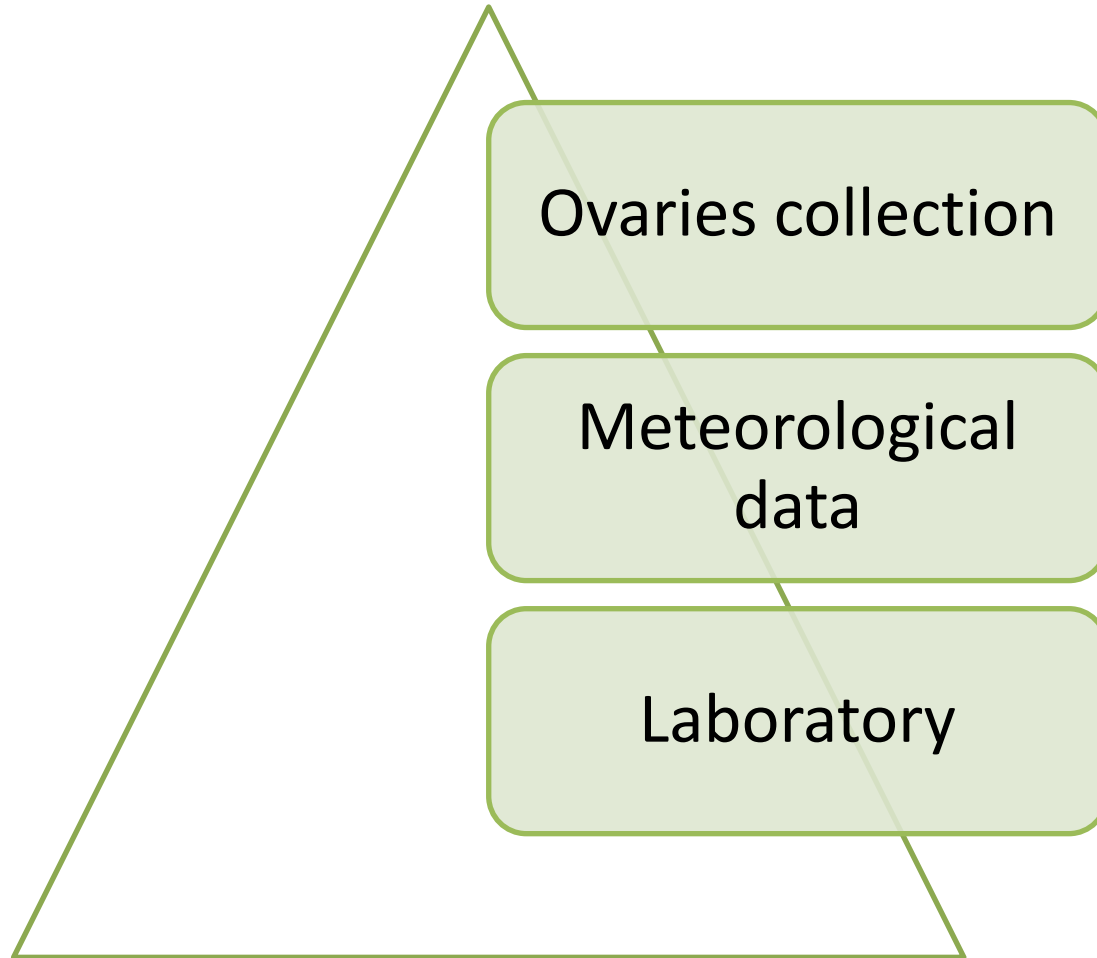
Selection (Abdalla et al., 2015).

Culture medium (Humblot et al., 2005; Harris and Picton, 2007).

Very few data (Kandil et al., 2014).

Objective

Breeding & non-breeding season

Two maturation media
- TCM-199
- MEM

Three types of serum
- FDCS
- BSA
- ESS

In-vitro maturation of camel oocyte

Material and methods

Ovaries collection

Meteorological data

Laboratory

Ovaries collection
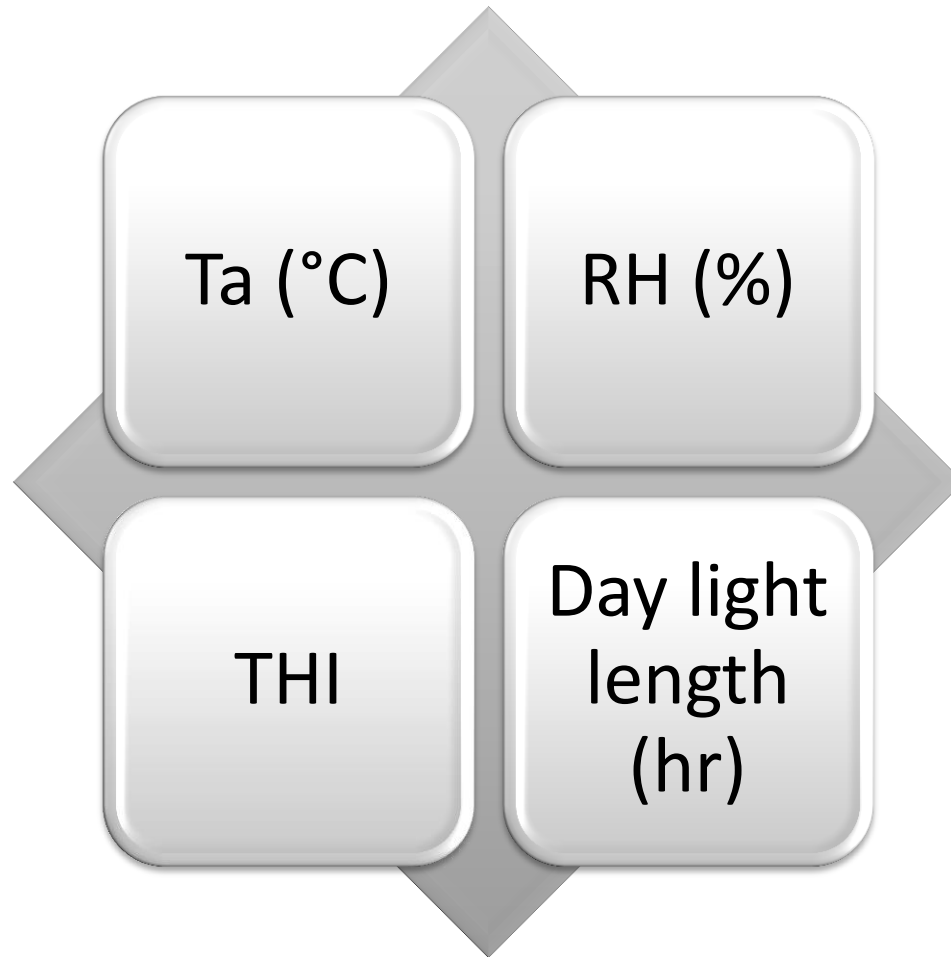
175 non-pregnant she-camel

350 ovaries

110 during breeding season

240 during non-breeding season

Meteorological data

Ta (°C)

RH (%)

THI

Day light length (hr)

Laboratory

Harvesting media (PBS)

Maturation media

Oocyte collection

Oocyte categories

Fixation

Staining

Examination for maturation

Statistical analysis

Oocyte categories

Compact cumulus oocyte

- Oocyte with five or more layers of complete cumulus cells.

Partial denuded oocyte

- Oocyte with cumulus cells present either incompletely surrounding the oocyte.
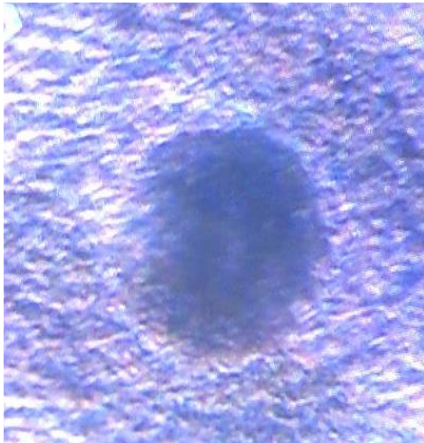
Denuded oocyte

- Oocyte without cumulus cells and covered by zona pellucida.

Shrunken (degenerated) oocyte

- Ooplasm shrunken away from the zona pellucida or not evenly filling the zona ooplasm looks degenerated with fragment empty zona pellucida.

Photomicrograph of the different oocyte categories.
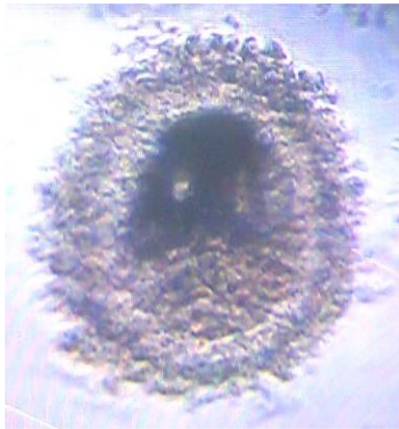

Compact cumulus

Partially denuded

Denuded oocyte

Degenerated oocyte

Treatment of maturation medium

The supplementation included the following media:

- T1: TCM-199 + 10% FDCS.
- T2: TCM-199 + 6mg/ ml BSA.
- T3: TCM-199 + 10% ESS.
- T4: MEM + 10% FDCS.
- T5: MEM + 6mg/ml BSA.
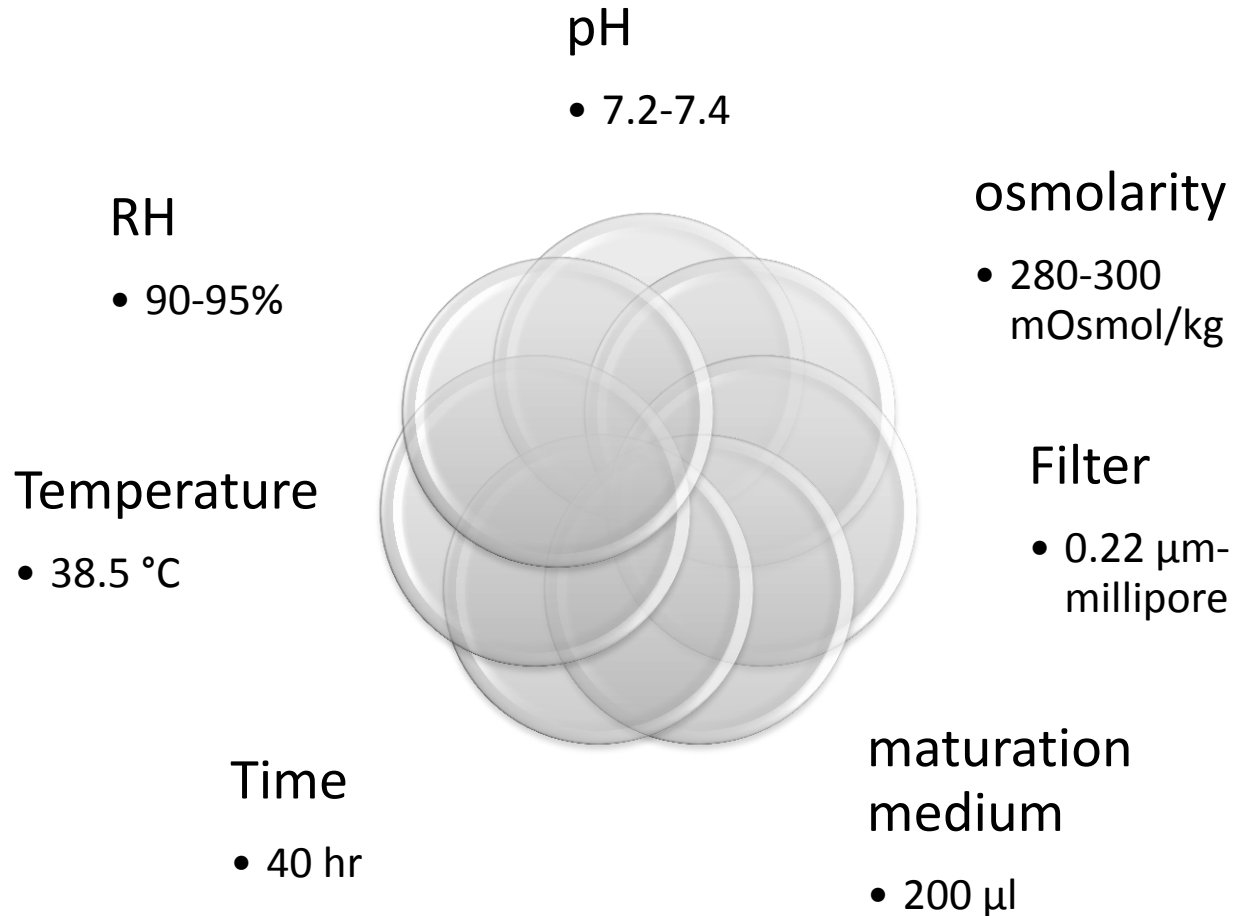- T6: MEM + 10% ESS.

Maturation

pH
- 7.2-7.4

osmolarity
- 280-300 mOsmol/kg

RH
- 90-95%

Filter
- 0.22 μm- millipore

Temperature
- 38.5 °C

maturation medium
- 200 μl

Time
- 40 hr

Table 1. Compositions of maturation media and sera

Content	Amount Per 100 ml
Media:	
TCM-199 or	**9 ml**
MEM (liquid)	**9 ml**
Sera:	
FDCS	**10%**
BSA	**6 mg/ml**
ESS	**10%**
PMSG	**20 µg/ml**
HCG	**10 IU/ml**
Oestradiol-17β	**1 µg/ml**
Na Pyruvate	**20 mMol**
Na Penicillin G	**100 IU/ml**
Streptomycin	**100 µg/ml**

Criteria of maturation

Germinal vesicle (GV)

- Inter phase chromosomes enclosed within a nuclear membrane.

Germinal vesicle breakdown (GVBD)

- An absence of a visible nuclear membrane and chromatin condensation characterized by a cluster of DNA material without individual chromosomes.

Metaphase-I (MI)

- Chromosomes were condensed in pairs and without detected polar body (immature oocytes).

Metaphase-II (MII)

- One the larger group of chromosome formed an equatorial plate and the remaining chromosomes are highly condensed or had extruded a polar body (oocytes mature).

Degenerated

- Oocytes were vacuolated or had scattered or highly condensed chromatin.

Results

Table 2. Air temperature, relative humidity, temperature-humidity index and length of day light, during breeding and non-breeding seasons.

Season	Air temperature (ºC)		Relative humidity (%)		Temperature-humidity index (THI)		Length of day light (hr)
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Breeding	11.23	21.66	43.02	58.49	50.80	68.02	12.24
Non-breeding	20.84	34.30	42.67	63.66	65.88	86.60	15.24

- No heat stress← 72 -moderate heat stress- 74 -severe heat stress- 78 → very severe heat stress

Table 3. Effect of breeding and non-breeding season on in-vitro maturation rate of camel oocytes.

Season	Total Oocyte number	Criteria of maturation									
		Germinal vesicle		Germinal vesicle breakdown		Metaphase-I		Metaphase-II		Degenerated	
		N	%	N	%	N	%	N	%	No.	%
Breeding	710	99	13.9	93	13.1	120	16.9	257	36.2[a]	141	19.9[b]
Non-breeding	650	86	13.2	90	13.9	111	17.1	217	33.4[b]	146	22.5[a]

[a] and [b]: Means denoted within the same column with different superscripts are significantly different ($P<0.05$).

Table 4. Effect of maturation media on in-vitro maturation rate of camel oocytes.

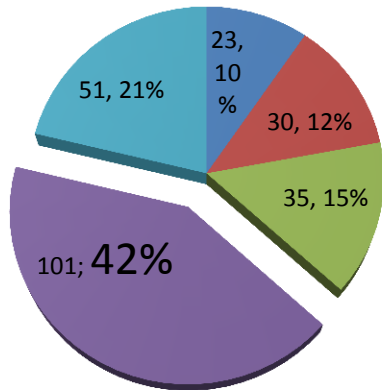| Type of medium	Total Oocyte number	Criteria of maturation									
		GV		GVB		Metaphase-I		Metaphase-II		Degenerated	
		N	%	N	%	N	%	N	%	N	%
TCM 199	700	87	12.4 [b]	90	12.9 [b]	116	16.7 [b]	253	36.1[a]	154	22.0 [a]
MEM	660	98	14.8[a]	93	14.1[a]	115	17.4[a]	221	33.5 [b]	133	20.1 [b]

[a] and [b]: Means denoted within the same column with different superscripts are significantly different ($P<0.05$). TCM199 = Tissue Culture Medium; MEM = Minimum Essential Medium

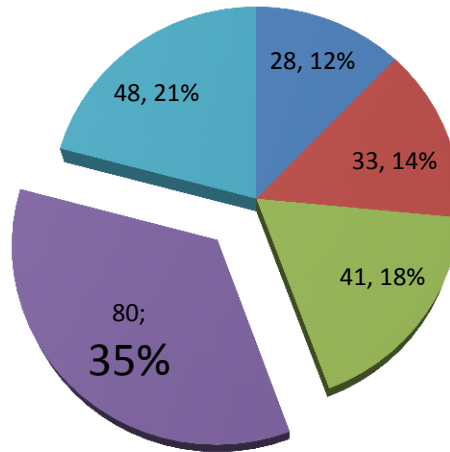## Table 5. Effect of type of serum on in-vitro maturation rate of camel oocytes.

Type of serum	Total oocyte number	Criteria of maturation									
		Germinal vesicle		Germinal vesicle breakdown		Metaphase-I		Metaphase-II		Degenerated	
		N	%	N	%	N	%	N	%	N	%
FDCS	465	52	11.2^b	61	13.1^a	70	15.1^b	190	40.8^a	92	19.8^b
BSA	455	64	14.1^a	65	14.3^a	83	18.2^a	152	33.4^b	91	20.0^b
ESS	440	69	15.7^a	57	13.0^a	78	17.7^a	132	30.0^C	104	23.6^a

[a] and [b]: Means denoted within the same column with different superscripts are significantly different ($P<0.05$). FDCS = Fetal Dromedary Camel Serum; BSA = Boivne Serum Albumin; ESS = Estrus Sheep Serum

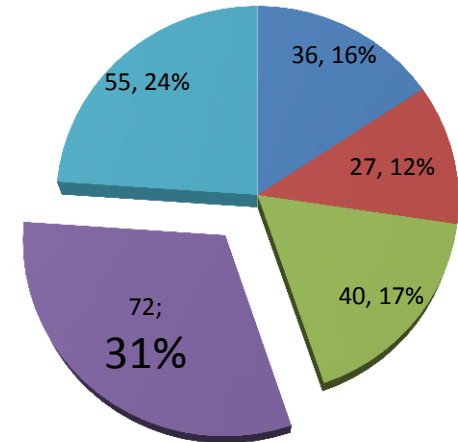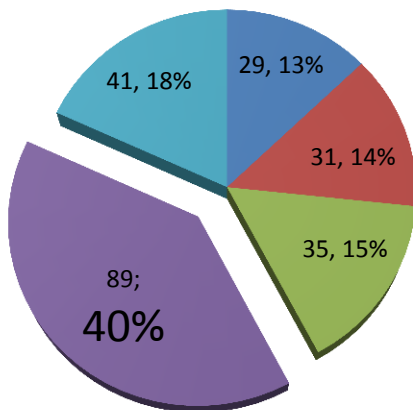**TCM 199 * FDCS**

- GV
- GVB
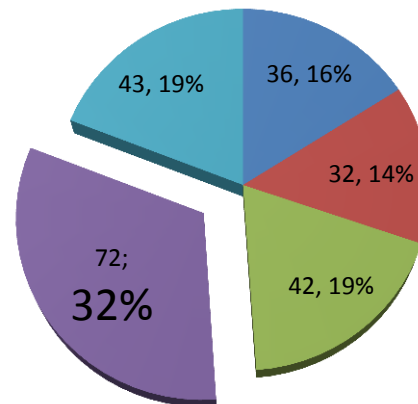- Metaphase I
- Metaphase II
- Degenerated

23, 10%
51, 21%
30, 12%
35, 15%
101; 42%

TCM 199 * BSA

28, 12%
48, 21%
33, 14%
41, 18%
80; 35%

TCM 199 * ESS

36, 16%
55, 24%
27, 12%
40, 17%
72; 31%

MEM * FDCS

29, 13%
41, 18%
31, 14%
35, 15%
89; 40%

MEM * BSA

36, 16%
43, 19%
32, 14%
42, 19%
72; 32%

MEM * ESS

33, 16%
49, 23%
30, 14%
38, 18%
60; 29%
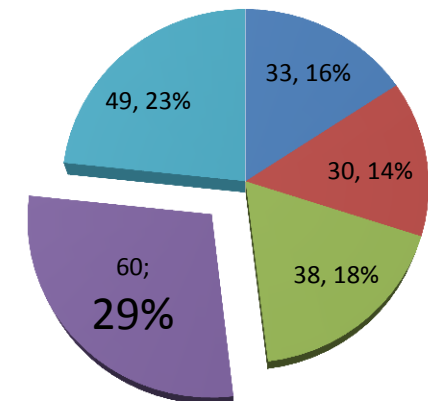
Conclusion

use of TCM-199 medium for in *vitro maturation* of camel oocytes added with fetal dromedary camel serum (FDCS) to promote *in vitro* camel oocytes maturation for in-vitro fertilization programme to enhance of fertilizing ability of she-camel oocytes, especially during breeding season.

Thank you for your attention