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### In Vivo and In Vitro Dromedary Camel Embryos' Production under Egyptian Conditions

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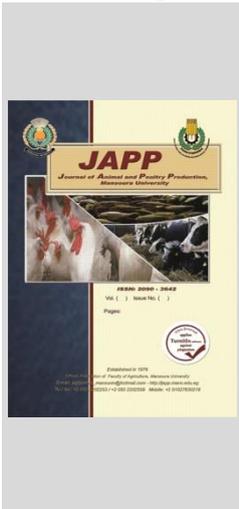
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#### ABSTRACT

The aim of the present study was to compare between *in vivo* and *in vitro* production of dromedary camel embryos under Egyptian conditions. At *in vivo* experiment, three female camels (donors) were treated with a one-shot injection of 3000 IU PMSG, followed by induction of ovulation treatment (i.m. administration of 5000 IU hCG). Natural mating was performed after 8 days from the superovulation treatment. Embryos were flushed via non-surgical collection after eight days of animal mating. For *in vitro* experiment the oocytes were retrieved by slicing ovaries collected from the slaughterhouse. Recovered oocytes were examined before and after IVM culture for grading and investigating cytoplasmic maturation. Only good-quality oocytes were selected (n=72). Cumulus oocytes complexes (COCs) were incubated at 38.5 °C, 5% CO<sub>2</sub> and 95% humidity for 40 hours. Matured COCs were inseminated with frozen-thawed semen (3x10<sup>6</sup> spermatozoa/mL) in the fert-TALP medium at 38.5 °C, 5% CO<sub>2</sub> and 95% humidity for 18 hours. Results showed that the *in vivo* embryos recovery rate was 74.92%. The hormonal treatment had no significant effect on the activity of ovaries regarding ovarian side. For *in vitro* experiment the mean oocyte yield was 9.83 COCs per ovary and the recovery rate (%) was 37.5%. The maturation rate of dromedary oocytes *in vitro* was 72.72%, while the fertilization rate was 11.5%. In conclusion, this study showed that *in vivo* embryo production can be considered as an effective tool for embryo in dromedary camels when compared to embryo production *in vitro*.

**Keywords:** Camels, dromedary, embryo production, IVF, IVM, oocyte, Superovulation, PMSG.



#### INTRODUCTION

Among the mammalian animals domesticated by humans for their needs, the camel has a distinctive status, being highly adapted to a specific ecosystem (the desert and harsh conditions worldwide) as it is a multipurpose animal used for production (milk, meat, wool, skin and manure), leisure, transport or agricultural work. No other domestic animal is able to provide such a variety of uses for human populations (Faye, 2016). According to FAO statics, the worldwide camel population is nearly 35 million heads (FAO, 2019). However, the reproductive performance of the one-humped camel is adversely affected by many complicated natural constraints (El-Hassanien *et al.*, 2004). Among these natural constraints, causing camels' low reproductive performance; delayed puberty, short breeding season, long gestation period, poor conception and high embryonic mortality (Tibary *et al.*, 2005; Skidmore and Billah, 2012; Hemeida, 2013, 2014). Early report by Wildt (1990) stated that assisted reproductive technologies (ART) have been developed to improve reproductive efficiency and genetics of animals, as well as for infertility treatment.

Embryo transfer (ET) is considered a valuable method to improve the rate of genetic progress, and exchange important breeding materials. It is also a method to more fully utilize the reproductive capacities of the most valuable females, and preservation of endangered species (Amstislavsky, 2006; Niasari-Naslaji *et al.*, 2009). Camel embryo transfer has seen massive development since 1990, and the interest for applying ET has been driven mainly by

the camel racing industry (Tibary *et al.*, 2005). Also, Skidmore and Billah (2005) reported two essential points for a successful implantation of an embryo transfer programs; the first point is a successful induction of superovulation in the donor females, specially that camels normally only carry a single fetus, this must be followed by an adequate synchronization program for the recipient females. Anouassi and Tibary (2013) reported the major gonadotropic hormones used for super-stimulation of ovaries in camels; like Pregnant Mare's Serum Gonadotropin (PMSG) and Follicle Stimulating Hormone (FSH). They noted that these gonadotropic hormones have been successfully used by many researchers with slight modifications of dosage in camels either during breeding season (Vyas *et al.*, 2004; Nowshari and Ali 2005; Azizi-Moghadam, 2010) or in nonbreeding season (Al-Sobayil, 2008). In 2010, Badr and Abdel-Malak reported that IVF technology in the dromedary camel will improve the understanding of the fundamental mechanisms of fertilization and early embryonic development and promote the development of other technologies.

Accordingly, the present study aimed to enhance the reproductivity of she-camels by recovering *in vivo* produced embryos for embryo transfer via a superovulation protocol (PMSG and GnRH) during breeding season in comparison with *in vitro* embryo production from ovaries of slaughtered animals.

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## MATERIALS AND METHODS

### 1. In vivo experiment:

The present experiment was carried out at Maryout Research Station, which belongs to the Desert Research Center (DRC), Ministry of Agriculture, located 34 km west of Alexandria. This experiment was performed during the breeding season (mid-January to mid-February 2017). The location is a semi-arid area. In the laboratory of Artificial Insemination and Embryo Transfer Lab (LAITE) that is well equipped with modern facilities for reproductive biotechnology applications.

### Animal feeding and management:

The female camels (n=3) used in the experiment were separately kept away from the main herd, while camel bulls, were kept in individual separate pens to avoid fetal fights between males during the rutting season. All animals were fed daily at 9 a.m. on a pelleted concentrate feed mixture with 14% crude protein content. The concentrates were supplemented with barley as a source of energy. Roughage as Berseem hay was also offered. Animals were allowed to drink twice daily and were offered 4 hours duration for free grazing.

### Animals:

Three dromedary she-camels with medium reproductive performance, averaging 10 years of age and 400 kg LBW were used as embryo donors in this study. Donors were selected from a 20 female camels' herd belongs to the DRC in Maryout Research Station. In addition, two fertile dromedary camel bulls, aged 15-17 years (average weight 550 kg) were used in the present study for natural mating of the females.

### Superovulation protocol of donors:

At the beginning of the hormonal protocol (0 day), each donor was treated with a one shot injection of 3000 IU PMSG (Folligon; Intervet Laboratories, Cambridge, UK) according to McKinnon and Tinson (1992). On day 8, ovulation was induced by i.m. injection of 5000 IU hCG (Chorulon, ITERVET, MSD, France) according to Manjunatha et al. (2012).

The experimental design and hormonal protocol of donors are summarized in Figure (1).

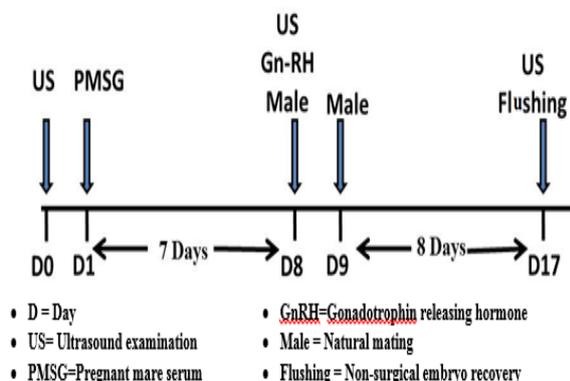


Figure 1. Experimental design and hormonal protocol for donors.

### Ultrasound examination and ovarian parameters

Ovarian follicular dynamics in she-camels were monitored using a Dynamic Imaging Concept MLV scanner ultrasound device (Eickemeyer Magic 5000 Digital, China),

integrated with a dual frequency (5.0–7.5 MHz) linear array probe. During the examination sessions, the animals were secured in a crush designed especially to keep them in a standing position. Both the number and size of follicles were recorded.

Donors were examined on 0 and 8 day of the protocol period to show number and diameter of large follicles on both right and left ovary of each donor (Figure, 1).

### Animal Mating:

At day eight, most follicles reached a mature size of 0.9–1.8 cm in diameter, the donors were individually introduced to the male for natural mating, each female was mated twice, 24 h apart, to one of two mature males.

### Non-surgical collection of embryos and embryo evaluation:

The donors (n=3) were flushed non-surgically in a standing position on day 8 post-mating (day 17 of the protocol period) as detected by Tibary and Anouassi (1997). Briefly, the vulva and surrounding perineal area were cleaned with sterilized dry gauze and then were disinfected with alcohol 70%. The females were sedated with Xyla-ject 2%. Thereafter, an 18 gauge Foley catheter (Bioniche, Canada) was introduced into the base of the uterine horn by the technique of intra-vagina. The cuff of the Foley catheter was inflated with 30 to 40 mL air and the uterine horn was flushed repeatedly with 30 to 70 mL Ringer solution as flushing medium. A total of 2000 ml mL flushing medium was used for each donor. By gravity, the flushing medium was recovered through Y tubing into a sterile embryo filter (Bioniche). The presence of embryo(s) was detected by examination of the residual filtrate (Figure, 2) in a gridded searching plate under a stereomicroscope (GX microscope, UK, Range: 8-50x)

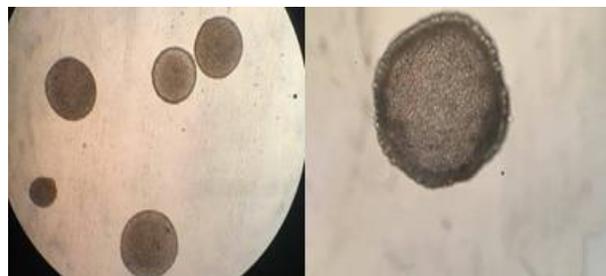


Figure 2. Camel embryo(s) recovered after flushing.(8 days)

### 2. In vitro experiment:

This experiment was conducted at the Embryology Manipulation Unite (EMU), Department of Animal and Poultry Physiology, Division of Animal and Poultry Production, Desert Research Center (DRC) Cairo, Egypt.

### Biological material

Camel (*Camelus dromedarius*) ovaries (n=12) of unknown reproductive history as the source of oocytes were collected from El-Bassatin slaughterhouse located in Cairo in a thermos flask containing warm normal saline solution (NSS, 0.9% NaCl, 35 to 37°C) supplemented with antibiotic antimycotic (100 µg streptomycin/ml and 100 IU penicillin) and transported to the laboratory within 2-3 hrs. While, frozen semen was obtained from artificial insemination and embryo transfer lab – Maryout Research Station – Desert Research Center, Alexandria, Egypt.

### Ovaries manipulation, oocyte retrieving and in vitro maturation

As soon as the camel ovaries reached the (EMU), all excessive tissues were discarded, and the ovaries were washed three times with pre-warmed (30 °C) NSS. Afterwards, all ovaries were washed once with ethanol (70%) to get rid of any contamination on the ovarian surface followed with a final wash using pre-warmed (30°C) phosphate buffer saline (PBS) supplemented with antibiotics (100 IU penicillin and 100 µg streptomycin/mL) according to Russo *et al.*, (2014). Then after, the ovaries were preserved in glass gars containing PBS and kept in a 30°C adjusted water bath (El-Sayed *et al.*, 2015).

Cumulus oocytes complexes (COCs) were harvested by slicing ovaries in a 90 mm petri dish and rinsing with warm (30°C) phosphate-buffered saline (PBS) supplemented with 50 µg/mL gentamicin (El-Sayed *et al.*, 2015). The recovered solution containing COCs was examined under a stereomicroscope (GX microscope, UK, Range: 8x to 50x) to pick out, evaluate and classify as good-quality (more than one layer of cumulus cells and homogeneous dark cytoplasm) according to the criteria described by Kandil *et al.*, (2014). Selected oocytes were washed twice with TCM 199 supplemented with 25 mM HEPES, 5% FBS then finally washed in IVM medium. Collected oocytes were cultured in IVM medium consisted of tissue culture medium-199 (TCM-199) supplemented with 15% (v/v) heat-treated (56 °C for 30 min) fetal bovine serum (FBS), 40 IU PMSG, 1 µg/mL estradiol (E2), 0.25 mg/mL Na<sup>+</sup> pyruvate, 20 ng/mL epidermal growth factor (EGF) and 100µg/mL ascorbic acid as antioxidant . All media were sterilized using 0.22µm millipore syringe filters and incubated for at least two hours in a 35 mm petri dish, covered with mineral oil and incubated

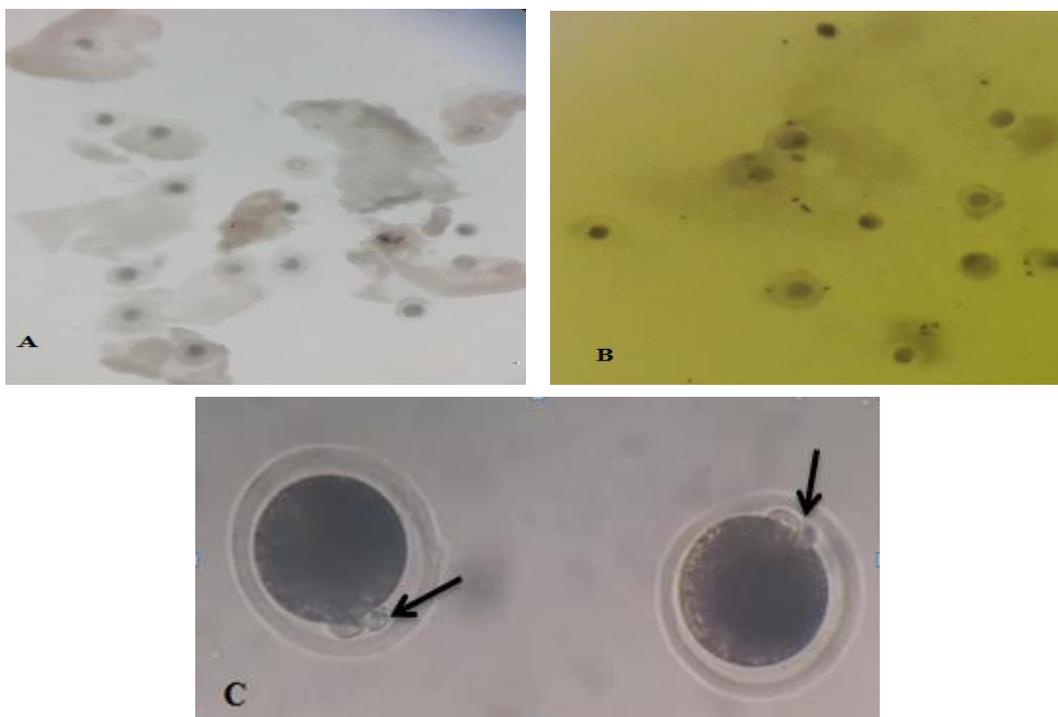
in CO<sub>2</sub> incubator for 40hrs at 38.5°C under 5% CO<sub>2</sub> and 95% relative humidity (RH). according to Russo *et al.*, (2014).

Oocytes were cultured in drops of maturation media (15 to 20 oocytes/ 100 µL drop) (Figure 3, A) Cumulus expansion was examined under stereo microscope (GX microscope, UK, Range:8x to 50x) after the incubation period. Percentage of oocytes with expanded cumulus (Figure 3, B) was calculated and recorded based on the criteria of Amer and Moosa (2009).

### In vitro fertilization

Two 0.5 mL straws of frozen camel semen were thawed (37°C for 40 seconds (El-Bahrawy, 2017) and wiped with 70% ethyl alcohol before being opened. and the contents were layered on top of 5 mL of sperm washing medium (Sperm TALP medium) in a 15 mL falcon tube and then, centrifuged twice at 300 X g for 5 min each. The final pellet was re-suspended in an appropriate volume (depending on the sperm concentration after washing) of pre-warmed fertilization medium (Fert. TALP medium). Then the final sperm concentration was adjusted to 3x10<sup>6</sup>/mL (El-Sayed *et al.*, 2015).

Three drops of 100 µL sperm suspension were placed in a 35 mm petri dish and covered with pre-warmed mineral oil. Matured oocytes were washed three times in the fertilization medium. 10 – 15 oocytes were placed in each of the sperm drops and cultured in a CO<sub>2</sub> incubator (Khatir *et al.*, 2007) for 18hrs at 38.5°C under 5% CO<sub>2</sub> and 95% RH. After fertilization, oocytes were denuded from cumulus cells by gentle pipetting in fertilization medium then observed with a Leitz Fluovert FU (Leica Microsystems, Wetzlar, Germany). Fertilization rate was calculated based on the observation of the second polar body (Figure 3, C).



**Figure 3. Developmental stages of *in vitro* oocyte maturation and fertilization showing: (A) immature oocytes with the condensed cumulus cells (40X), (B) matured oocytes with expanded cumulus cells (40x) and (C) fertilized oocyte extruded the 2<sup>nd</sup> polar body (black arrows, 300x).**

**Statistical analysis**

Data were expressed as mean ± SE for follicles number, follicle size, number of recovered embryos, embryos recovery rate, expansion rate, number of fertilized oocytes and fertilization rate.

**Effect of ovarian side on total follicles number and average follicular size of superovulated she-camels was performed using SPSS 13.0 (SPSS Inc, Chicago, Illinois). A test of significance was conducted using t-test.**

**RESULTS AND DISCUSSION**

A total of 19 (Day 8) embryos were recovered from the uteri of 3 super ovulated female dromedary camels. For all treated females, the effect of the hormonal treatment had no significant effect on the ovarian activities depending on the ovary position (right or left). Whereas, the total follicles number was with an overall of 4.33 follicles and the average size of the mature follicles was (1.22 cm) as shown in Table (1). In general, as shown in Table (2), the recovery rate for the embryos was 74.92% for recovered embryos compared to the number of detected follicles.

**Table 1. Effect of ovarian side on total follicles number and average follicular size of superovulated she-camels. (mean ± SE)**

Ovarian side	Total follicles number/ovary	Average follicular size (cm)
Left	4.67 ± 0.33	1.13 ± 0.06
Right	4.00 ± 1.53	1.31 ± 0.07
t-value	0.426	-1.97
P-value (Sig.)	0.692 <sup>(n.s.)</sup>	0.694 <sup>(n.s.)</sup>
Overall mean	4.33 ± 0.71	1.22 ± 0.06

**Table 2. Effect of superovulation on follicles number, recovered embryos and embryos recovery rate (%)**

Animal No.	Total Follicles (No)	Recovered Embryos (No)	Embryos Recovery Rate (%)
1	11.00	7.00	63.64
2	9.00	7.00	77.78
3	6.00	5.00	83.33
Overall mean ± SE	8.67±1.45	6.33±0.67	74.92±5.86
t-value	5.96	9.5	12.78
Significance	0.03	0.01	0.01

p < 0.05

The follicles size after 8 days from the PMSG treatment were in range between 0.9 to 1.81 cm, these findings were similarly reported by other authors, when eCG (2000 to 3000 IU) is injected to females with no dominant follicle, the interval from treatment to mating (follicular diameter of 1.2 cm) is relatively constant (8 days) (Tibary and Anouassi, 1997 and Tibary et al., 2007). For superstimulated animals, the mean number of collected embryos was 7.1 ± 4.3 (range 0-19) for eCG-treated females. with about 20% of the females not responding (Anouassi and Ali, 1993). However, natural mating or artificial insemination is performed when follicles are between 1.2 and 1.6 cm in diameter and breeding should never be delayed beyond the 20 mm follicular size because of the increased incidence of failure of luteinization or ovulation at this stage (Vaughan et al., 2013).

Table (2) showed an overall mean of 6.33±0.67 recovered embryos with significant difference between the

three different females and the overall mean in recovered embryos and recovery rate. in this experiment, our results are closed to that previously reported by, Skidmore et al.,(2002) who reported that, total of 242 embryos were recovered from uteri of 36 adult dromedary camels that flushed non-surgically 7 days after ovulation, the mean ± SE number of embryos recovered per flushing attempt was 7.8±1.4 (range 1–22). Vettical et al., (2016) reported that, donors were then screened and mated approximately 10 days later when the major follicles had reached a mature size of 1.3–1.7 cm in diameter, they observed the mean ± SE number of follicle development, ovulation, total embryo recovery and useable quality embryo recovery of (12.75 ± 2.93), (9.13 ± 3.64), (8.50 ± 3.27) and (6.25 ± 2.47), respectively. Most recent studies by Abd-Elfattah et al., (2020) recovered 487 embryos from 56 female that had been super-ovulated and mated with a mean of 8.7 embryos/ female. The results here in this investigation surpassed what have been achieved through Vyas et al., (2004) who noted that a considerable individual variation in response to the stimulation of superovulation was observed, the uteri of 17 donors were flushed end gave 30 embryos (1.51 embryos/donor), later, Skidmore and Billah, (2011) reported that 42 embryos were recovered from the uteri of 12 mated donor camels by non-surgical flushing 7 days after ovulation.

In *in vitro* experiment, total number of 118 oocyte (72 good quality and 46 bad quality) recovered from 12 dromedary she camels’ ovaries. The average of total number of camel oocytes recovered per camel ovary (oocyte yield /ovary) was 9.83 COCs/ camel ovary (table, 3). Which is similar to El-Sayed et al., (2012) being 9.4% but lower than Kandil et al., (2014) and Abdel–Khalek et al., (2010) being 10.85, 12.4 respectively. On the other hand, it was higher than Mahmoud et al., (2003), Ali and Abdel–Razek, (2001) being 5.3, 6.67 respectively. Recovery rate of oocytes/ follicles (%) recorded 37.5% which is close to the result of El-Harairy et al., (2007) being 37.71%.

**Table 3. Recovery rate of dromedary camel oocytes**

	Oocyte yield/ Ovary	Follicular number /ovary	Oocyte recovery rate
mean±SE	9.83±1.91	26.08±3.29	37.5±4.94

The expansion rate (%) (Table, 4) of dromedary camel oocytes *in vitro* maturation was 72.72%, which is close to the result of Wani and Wernery (2010) and Elsayed et al., (2015) ( 67-71%, 71.5% respectively), but it is higher than that of El-Sayed et al., (2012) and Mesbah et al., (2016) (68%, 68.49% respectively). These differences in results may be attributed to differences in age and season, reproductive status, site of the ovary, method of oocytes collection and periods of ovary preservation.

The fertilization rate of dromedary camel oocytes was 11.51% that is lower than the result obtained by Khatir et al., (2007), Khatir and Annossia (2006), Kamel (2015), El-Sayed et al (2015) and Fathi et al., (2014) ( 68%, 64%, 20.7%, 19% and 17% respectively). This decrease in fertilization ratio could be due to several factors such as different length of maturation period (40 h) and use of frozen thawed semen in fertilizing oocytes.

The huge gap between *in vivo* embryo production (74.92%) and *in vitro* embryo production (11.51%) may be due to that oocytes resume meiosis *in vivo* when the

dominant follicle reaches a size more than 1cm in dromedary (Tibary and Anouassi, 1997) which mean that fertilization occurs at the optimum time. Regardless, that the other conditions that affect embryonic development are at optimum levels. While, Oocytes used for *in vitro* maturation of dromedary camel are usually collected from different population of follicles about 2–10mm derived from slaughtered animals with unknown reproductive history (Abdoon, 2001; Torner *et al.*, 2003; Kafi *et al.*, 2005; Khatir *et al.*, 2005). Additionally, optimum duration of IVM is 36 h for dromedary camel (Abdoon, 2001; Kafi *et al.*, 2005; Khatir *et al.*, 2005; Khatir and Anouassi, 2006; Torner *et al.*, 2003). This is significantly accelerated considering that in the dromedary camel, ovarian follicular growth from 0.2 cm to a size able to ovulation response (>0.9 cm) requires nearly 5–6 days. (Tibary and Anouassi, 1997). During the final follicular maturation, important changes in oocyte nuclei occur that may have a crucial effect on the obsession of oocytes developmental competence. In addition, the low rate of *in vitro* fertilization may be due to that the cytoplasmic maturation of oocytes was incomplete (72.72%) and/or to the Insufficient *in vitro* culture system. This suggestion is according to Rizos *et al.*, (2002) who found that oocytes matured *in vivo* are more competent than those matured *in vitro*, also the quality of the oocyte is the main factor affecting blastocyst yields, while the embryo culture conditions have an important role in determining the quality of the blastocyst.

**Table 4. *In vitro* fertilization of dromedary camel oocytes**

Replicate No	No of Oocytes in Replicates	Expanded Oocytes (No)	Expansion Rate (%)	Fertilized Oocytes (No)	Fertilization Rate (%)
1	13	10	76.92	4	30.77
2	19	15	78.95	1	5.26
3	20	15	75	1	5
4	20	12	60	1	5
Overall mean ± SE	18±1.68	13±1.23	72.72±4.32	1.75±0.75	11.51
t-value	10.69	10.61	16.85	2.33	1.79
Significance	0.02	0.02	0	0.1	0.17

p < 0.05

Also, a major factor could be the difference between natural mating which applied in *in vivo* embryo production in comparison with IVF using frozen thawed semen. This is suggested according to the study conducted by Al-Bulushi *et al.*, (2019) who found that pregnancy rate in dromedary camel strongly affected by applying natural mating or AI with a whole fresh undiluted semen (83.3% and 81.8%, respectively) or applying fresh diluted semen which ranged from 23 to 70% depending on extender, concentration, timing, and site of insemination. Moreover, they also found that using chilled semen reduced the pregnancy ratio to 11.1% - 21.1% depending on the type of extender. While applying frozen semen produced no pregnancy at all.

## CONCLUSION

*In vivo* embryo production can be considered as an effective tool for promoting the fertility potential in dromedary she-camels. On the other hand, and despite its low ratio but *in vitro* embryo production considers one of the most important methods of enhancing reproductivity of

dromedary camel and further studies are needed to optimize its results.

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### Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

### Authors' contribution

Prof. Dr. Khalid Ahmed El Bahrawy: Field and laboratory work supervision, designed the experiment, manuscript writing and final revision. Prof. Dr. Nehal Ali Abu Elnaga designed the experiment and revised the article. While Mrs. Amira Khaled Khattab participated in laboratory work, analysis and manuscript writing. All authors have read and approved the final manuscript.

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## إنتاج أجنة الإبل وحيدة السنام حقلياً و معملياً تحت الظروف المصرية

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الهدف من الدراسة الحالية هو المقارنة بين إنتاج الأجنة حقلياً و معملياً للإبل وحيدة السنام تحت الظروف المصرية. في التجربة الأولى، تم معاملة ثلاثة إناث (مانحين) بحقن جرعة واحدة ٣٠٠٠ وحدة دولية PMSG، يليها دفع هرموني للتبويض (حقن عضلي ل ٥٠٠٠ وحدة دولية hCG). تم تلقيحهم طبيعياً بعد ٨ أيام من دفع التبويض. وتم إسترداد الأجنة عن طريق الجمع غير الجراحي بعد ثمانية أيام من التلقيح الطبيعي. بالنسبة للتجربة الثانية، تم إسترداد البويضات عن طريق تشريح المبايض التي تم جمعها من المسلخ. وبعد ذلك تم فحص البويضات المتحصل عليها قبل وبعد الزراعة في بيئة الإنضاج المعملية من أجل تصنيف وتحديد مدى النضج السيتوبلازمي. تم اختيار عدد ٧٢ من البويضات عالية الجودة فقط. تم تحضين البويضات الغير ناضجة عند ٣٨,٥ درجة مئوية و ٥ ٪ ثاني أكسيد الكربون و ٩٥ ٪ رطوبة لمدة ٤٠ ساعة. تمت عملية الإخصاب المعملية عن طريق تحضين البويضات الناضجة مع السائل المنوي المجمد بعد إسالته على ٣٧ درجة مئوية لمدة ٤٠ ثانية وضبط التركيز ليكون (٣ × ١٠<sup>٦</sup> حيوان منوي / مل) في بيئة الإخصاب TALP عند ٣٨,٥ درجة مئوية و ٥ ٪ ثاني أكسيد الكربون و ٩٥ ٪ رطوبة لمدة ١٨ ساعة. أظهرت النتائج أن معدل إسترداد الأجنة في التجربة الحقلية كان ٧٤,٩٢ ٪. لم يكن للمعاملة الهرمونية تأثير معنوي على نشاط المبيض. بالنسبة للتجربة المعملية، كان متوسط عدد البويضات المستردة ٩,٨٣ بويضة لكل مبيض وكان معدل الإسترداد ٣٧,٥ ٪. بلغ معدل نضج البويضات اعتماداً على تمدد الخلايا المحيطة بها ٧٢,٧٢ ٪، بينما كان معدل الإخصاب ١١,٥ ٪. وبناءً على ما سبق، فقد أظهرت هذه الدراسة أن إنتاج الأجنة حقلياً يمكن اعتباره أداة فعالة لتحسين الكفاءة التناسلية في الإبل وحيدة السنام عند مقارنتها بإنتاج الأجنة معملياً.