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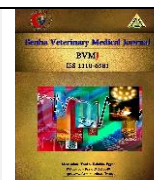
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Original Paper

In vitro maturation (IVM) of camel oocytes using different maturation media.

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ABSTRACT

The aim of the present study is to evaluate the in vitro maturation rate of camel oocytes using different maturation media. Ovaries of 50 she-camels were collected during the breeding season (from December to April). Ovaries were transferred to the lab in a thermal flask containing warm saline solution within 2 hrs. After slaughtering. Oocytes were collected using 2 techniques; aspiration of graafian follicles (2-5mm size) and slicing of the ovaries. Depending on the morphological quality, the collected oocytes were selected and cultured in different types of maturation media (GTL, HAM'S1, HAM'S 2, HAM'S 3) for 30-40 hours at 38.5˚c, 5% CO_2, and maximum humidity (95-96%). The obtained results revealed that the slicing technique produced a higher oocyte recovery rate compared to the aspiration technique. In addition, the maturation media composition mainly the FSH affects the in vitro maturation rate of camel oocytes. It could be concluded that the type of oocyte maturation media might be one factor that improve the rate of oocyte maturation.

1. INTRODUCTION

Camel plays an important part in the agricultural matrix, especially in desert areas, providing milk, meat, and transportation (Skidmore, 2005). Camel is popular in Egypt, particularly in the meat production sector (Farah and Fischer, 2004). Camel reproductive efficiency has been reported to be low in natural pastoral conditions due to the short breeding season, late onset of puberty, early embryonic mortality, and long calving intervals (El-Wishy, 1987; Marai et al., 2009). Camel reproductive efficiency is highest in Egypt from December to April (Basiouni, 2007), which has a direct impact on embryo production capacity (Amer and Moosa, 2009) The use of assisted reproductive technologies like artificial insemination, embryo transfer, and in vitro embryo production, as seen in most livestock species, could provide a better understanding of the variables that governs reproduction in this species. Using an in vitro embryo production technique may aid in maximizing the number of embryos obtained. However, such a technique is still not widely used in camels (Skidmore, 2005). Numerous trials were carried out to investigate the viability of in vitro oocyte maturation in camelids as well as dromedary camels (Abdoon, 2001; Torner et al., 2003; Khatir et al., 2004; Nowshari and Wani, 2005; Khatir and Anouassi, 2006). In this regard, the implementation of in vitro embryo production technology can assist in the investigation of fundamental mechanisms governing reproduction in camels. A large number of high-quality oocytes are needed for the In vitro maturation technique (IVM), which is dependent on the ovary's varying number of follicles as well as the manner of recovery (Zeidan et al., 2011). In addition to influencing the proportion of oocytes capable of maturation, oocyte maturation regulation also had an impact on how many oocytes would fertilize and develop after maturation (Bavister et al., 1992). Camel oocyte maturation and in vitro fertilization have not been extensively studied. It was thought that conditions present during in vitro maturation, embryo culture, and fertilization were essential for assisting embryos in acquiring development competence. (Alvarez et al., 2013). The quality of oocytes and embryos are affected by different factors including temperature, time, culture media characteristics, and oxidative stress, which has stated as one of the major contributors to poor quality of in vitro produced embryos (Karja et al., 2004).
Therefore, the purpose of this study is to ascertain the impact of various maturation media on the in vitro maturation process of camel oocytes.

2. MATERIAL AND METHODS

The present study was carried out in the laboratory of Fertility and Assisted Reproductive Technology Unit, Obstetrics and Gynecology Hospital (Cairo University). Ethical Approval No. BUFVTM 08-11-22.

2.1. Samples:
A total number of 100 ovaries collected from 50 she-camels during the breeding season (from December2019 to

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April 2020) have been used in this study. The ovaries were collected from El-Warraq abattoir in Giza and El-Bassatin abattoir in Cairo. According to Sghiri and Driancourt (1999); Basiouni, (2007); Tibary et al. (2007), the ovaries were transferred to the laboratory within 2 hours in a thermal flask with pre warmed saline solution (SS, 0.9% NaCl) supplemented with gentamicin kept at 30-35˚c. In the lab, ovaries were terminated, washed twice with warm SS, once with ethyl alcohol (70%) quickly and two additional times with warm SS (Khatir and Anouassi, 2006).

2.2. Oocyte collection:

Oocytes were extracted from the ovaries using 2 different techniques; aspiration and slicing techniques. In the aspiration technique (286 oocytes), according to Ali and Abdel-Razek (2001), graafian follicles of 2-5 mm size have been aspirated using 18- gauge needle attached to 5 ml plastic syringe containing 0.5 ml phosphate buffered saline (PBS) supplemented with fetal calf serum (FCS, 10%) and gentamicin (5µl/ml). In the slicing technique (461 oocytes), the ovaries underwent a complete slicing by using a sterile scalpel in 60 mm petri dish which contained phosphate buffered saline (PBS) supplemented with 5µl/ml gentamicin and 10% FCS. The slicing fluids were collected and transferred into another clear 60 mm petri dish (Swain and Pool, 2012).

The follicular fluid and flushing media have been examined under stereomicroscope for oocyte collection. The number of oocytes collected from each technique was calculated and the oocyte recovery rate (ORR, %) was determined as the following:

ORR (%) = (Number of recovered oocytes/ total number of follicles) x 100.

2.3. Oocyte selection:

According to Fathi et al. (2017), the retrieved oocytes were classified into four different grades (figure 1);

Grade 1: with more than three layers of cumulus cells.

Grade 2: oocytes were covered by one to three layers of cumulus cells.

Grade 3: oocytes were denuded.

Grade 4: oocytes with uneven dark ooplasm.

The oocytes with Grades 1 and 2 (figure 1) were selected and used for maturation (Nowshari and Wernery, 2003).

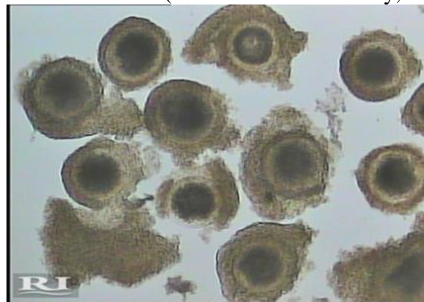

Figure 1 Different grade of camel oocytes.

2.4. In vitro maturation:

In the present study 4 types of maturation media were used as follow;

1. GTL media (a bicarbonate buffered medium containing hyaluronan and human serum albumin for culture of embryos from fertilization to blastocyst stage and for transfer, 10145, VitroLife, Sweden).
2. HAM'S 1 consists of HAM'S F10 with FCS (10%), gentamicin, pregnant mare serum (PMS) (0.01 %) and follicular stimulating hormone (FSH, 75 IU) as (Zeidan et al., 2008).
3. HAM'S 2 consist of HAM'S F10 with FCS (10%), gentamicin, PMS (0.01%) and FSH (150 IU) (Zeidan et al., 2008).
4. HAM'S 3 consists of HAM'S F10 with FCS (10%), gentamicin, PMS (0.01%), FSH (75 IU) and luteinizing hormone (LH, 75 IU) (Zeidan et al., 2008).

The selected oocytes (N= 600 oocytes) were washed twice in PBS and once in a pre-incubated maturation media and cultured in the four different maturation media (150 oocyte /type of media). The oocytes were cultured in drops of a preincubated 50 µl maturation media (5 oocytes/drop) covered with mineral oil in 35 mm petri dish. The cultured oocytes were incubated for 30-40 hrs at 38.5˚c, 5% CO_2 and maximum humidity (96%), after which the oocytes were evaluated for maturation. Maturation was assessed by determination of cumulus cells mass expansion and or the presence of first polar body (PB) (figure 2) (Schellander et al., 1990).

Figure 2 Mature oocyte (notice appearance of the first polar body).

2.5. Statistical Analyses:

Data were analyzed by Chi-square test using SPSS (version 25) software. The statistical significance was set at $P<0.05$.

3. RESULTS

As presented in table 1, a significant ($P < 0.000$) difference in the oocyte recovery rate has been recorded between the different oocyte collection techniques. The total number of retrieved oocytes was 747 of which 461(62%) were collected by the slicing technique, while 286 (38%) oocytes were collected by the aspiration technique.

The effect of different maturation media on the in vitro maturate rate of camel oocyte is shown in table 2. A significant ($P < 0.05$) differences have been recorded between the different groups of maturation media and the highest level of maturation was recorded with the groups HAM'S 1 (56%) and HAM'S 2 (68%) media. No difference was found between G-TL and HAM'S 3 media regarding the percentage of in vitro matured oocytes.

Table 1 Effect of oocyte collection methods on the number and recovery rate (%) of camel oocyte.

Item	Aspiration	Slicing	Total	Sig.
No of ovary	50	50	100	
No. of recovered oocytes	286^{b}	461^{a}	747	0.01
Oocyte recovery rat (ORR, %)	38^{b}	62^{a}	100	0.01

Values with different letters within the same row differed significantly at $P < 0.01$.

Table 2 The number and percentage of matured oocytes according to the type of media.

Type of media	Number of cultured oocytes	Number of matured oocytes	Percentage of matured Oocytes (%)
G-TL	150	60	40[a]
HAM'S 1	150	84	56[b]
HAM'S 2	150	102	68[c]
HAM'S 3	150	60	40[a]

4. DISCUSSION

Oocyte recovery procedures have a significant impact on the number and quality of recovered oocytes, which plays an important role in the in vitro produced embryos. Comparing the two techniques used in this investigation, ovarian slicing produced a higher rate of oocyte recovery. Our findings come in agreement with that reported by Swain and Pool (2012) and AL-Fatlawy and Hussain (2018). In the same manner, the slicing technique was reported to produce a higher oocyte recovery rate in buffalo (Gasparrini, 2001; Rao and Mahesh, 2012; Hammad et al., 2014) and cattle (Zheng et al., 2007) compared to the other technique of oocyte retrieval. Contrary to our findings, aspiration rather than slicing was found to generate more oocytes in camels (Purohit et al., 1990; Abdelkhalek et al., 2017) and buffalo (Khan et al., 1997). The release of oocytes from both surface follicles and deeper cortex, as well as the loss of some during follicular aspiration which is not feasible in the slicing procedure, have been suggested as probable causes of the increased oocyte recovery rate attained by slicing compared to aspiration technique (Das et al., 1996; Hammad et al., 2014).

The in vitro maturation rate of camel oocytes was evaluated in this work under the influence of various maturation media with varying compositions, primarily the presence and concentration of FSH. By measuring the mass expansion of cumulus cells and/or the existence of the first polar body (PB), maturation was evaluated (Schellander et al., 1990). The current findings showed that the in vitro maturation rate increased in media containing only FSH as well as when the concentration of FSH was increased. Our justification is based on the findings from Eppig (1979a and b) and Pandey et al. 2010, which show that FSH is the main hormone driving cumulus cell expansion.

5. CONCLUSION

It could be concluded that the number of collected oocytes depends on the methods of oocytes collection. In addition, the modification of maturation media might be one of the factors that improve camel oocyte maturation.

6. REFERENCES

1. Abdelkhalek, A.E, Gabr, Sh. A., Khalil, W.A., Shamiah, Sh.M., Pan, L., Qin, G., Farouk, M.H., 2017. In vitro production of Sudanese camel (Camelus dromedarius) embryos from epididymal spermatozoa and follicular oocytes of slaughtered animals. Polish Journal of Veterinary Sciences, 20(1), 95–101.
2. Abdoon, A.S.S., 2001. Factors affecting follicular population, oocyte yield and quality in camels (Camelus dromedarius) ovary with special reference to maturation time in vitro. Anim Reprod Sci, 66, 71-79.
3. AL-Fatlawy M.A. and Hussain, S.O., 2018. Assessment Of Different Techniques for Oocyte Collection for Ivf in the Camel (Camelus dromedarius). Kufa Journal for Veterinary Medical Sciences, 9(1), 76-88.
4. Ali, A. and Abdel-Razek, A.K.H., 2001. Comparison of number and quality of oocytes in the Egyptian buffaloes (Bubalus bubalis), cows (Bos taurus) and camels (Camelus dromedarius). Assiut Vet Med J, 45, 317–325.
5. Alvarez, G.M., Ferretti, E.L., Gutnisky, C., Dalvit, G.C., Cetica, P.D., 2013. Modulation of glycolysis and the pentose phosphate pathway influences porcine oocyte in vitro maturation. Reproduction Domest Animals. 48, 545e53.
6. Amer, H. and Moosa, A., 2009. Effect of season and culture media on the competence of dromedary camel oocyte to mature in vitro. Global Veterinaria, 3(1), 01-08.
7. Basiouni, G.F., 2007. Follicular wave pattern, folliculogenesis and assisted reproductive techniques in the non-pregnant female dromedary camel (Camelus dromedarius). J Biol Sci, 7(6), 1038- 1045.
8. Bavister, B.D., Rose Hellekant, T.A., Pinyopummintr, T., 1992. Development of in vitro matured and in vitro fertilized bovine embryo into morula and blastocysts in defined culture media. Theriogenology, 37, 127 – 146.
9. Das, G.K., Jain, G.C., Solanki, V.S., Tripathi, V.N., 1996. Efficacy of various collection methods for oocyte retrieval in buffalo. Theriogenology, 46, 1403-1411.
10. El-Wishy, A.B., 1987. Reproduction in the female dromedary (Camelus dromedarius): Anim Reprod Sci, 82, 587-593.
11. Eppig, J.J., 1979a. FSH stimulates hyaluronic acid synthesis by oocyte-cumulus cell complexes from mouse preovulatory follicles. Nature, 281, 483-484.
12. Eppig, J.J., 1979b. Gonadotropin stimulation of the expantion of cumulus oophori isolated from mice: general conditions for expantion in vitro. J Exp Zool, 208, 111-120.
13. Farah, Z. and Fischer, A., 2004. Milk and Meat from the Camel. Handbook on Products and Processing. vdf Hochschulverlag AG,ETH Zentrum, CH-8092 Zürich.
14. Fathi, M. and El-Shahat, K.H., 2017. L-Carnitine enhances oocyte maturation and improves in vitro development of embryos in dromedary camels (Camelus dromedaries). Theriogenology,104, 18-22.
15. Gasparrini, B., 2001. In vitro embryo production in buffalo species: state of the art. Theriogenology, 57, 237-256.
16. Hammad, M.E., Gabr, Sh.A., El-Ratel, I.T., Gad, M. A.2014. Efficacy of different collection techniques on yield and quality of Egyptian buffalo oocytes. J Animal and Poultry Prod, Mansoura Univ, 5(7), 413-422.
17. Karja, N.W., Wongsrikeao, P., Murakami, M., Agung, B., Fahrudin, M., Nagai T., 2004. Effects of oxygen tension on the development and quality of porcine in vitro fertilized embryos. Theriogenology.62:1585-1595.
18. Khan, I.Q., Samad, H.A., Rehman, N.U., 1997. Quantity and quality of buffalo follicular oocytes

recovered by aspiration and scoring methods for in vitro studies. Pak Vet J, 17, 187-189.
19. Khatir, H., Anouassi, A., Tibary, A., 2004. Production of dromedary (Camelus dromedarius) embryos by IVM and IVF and co-culture with oviductal or granulosa cells. Theriogenology, 62, 1175–1185.
20. Khatir, H. and Anouassi, A., 2006. The first dromedary (Camelus dromedarius) offspring obtained from in vitro matured, in vitro fertilized and in vitro cultured abattoir-derived oocytes. Theriogenology, 65, 1727-1736.
21. Marai, I.F.M., Zeidan, A.E.B., Abdel-Samee, A.M., Abizaid, A., Fadiel, A., 2009. Camel reproductive and physiological performance traits as affected by environmental conditions. Tropical and Subtropic Agroecosystems, 10, 129–149.
22. Nowshari, M.A. and Wernery, U., 2003. The effect of harvesting technique on efficiency of oocyte collection and their meiotic competence in dromedary camels. Ninteenth Meeting A.E.T.E. Rostoc 12-13. pp. 196.
23. Nowshari, M.A., and Wani, N.A., 2005. Camelid embryo development in vitro: effect of protein supplementation in maturation medium and subsequent culture in two different media on fertilization and development. Reprod Fertil Dev, 17, 276-282.
24. Pandey, A., Gupta, S.C., Gupta, N., 2010. Effect of FSH and LH hormones on oocyte maturation of buffalo and gene expression analysis of their receptors and Cx43 in maturing oocytes. Zygote 18, 321-234.
25. Purohit, G.N., Datt, M., Sharmn, S.S., 1990. Efficiency of three methods of oocyte recovery in the dromedary camel (Camelus dl'omedarius). Indian Journal of Animal Sciences, 69 (2), 88-90.
26. Rao, M.M. and Mahesh, U.Y., 2012. Efficacy of different harvesting techniques on oocyte retrieval from buffalo ovaries. Buffalo Bulletin, 31 (4), 209-213.
27. Schellander, K., Fuhrer, F., Brackett, B.G., Korb, H., Schleger, W., 1990. In vitro fertilization and cleavage of bovine oocytes matured in medium supplemented with estrous cow serum. Theriogenology, 33, 477-485.
28. Sghiri, A. and Driancourt, M.A., 1999. Seasonal effects on fertility and ovarian follicular growth and maturation in camels (Camelus dromedarius). Anim Reprod Sci, 55, 223–237.
29. Skidmore, J.A., 2005. Reproduction in dromedary camels: an update. Anim Reprod, 2(3), 161. 171.
30. Swain, J. and Pool, T., 2012. Culture media in IVF: Decisions for the laboratory. In: Nagy ZP, Varghese AC, Agarwal A. (Eds.). Practical Manual of In Vitro Fertilization: Advanced Methods and Novel Devices. Springer Science+Business Media, LLC, 79-90.
31. Tibary, A., Anouassi, A., Sghiri, A., Khatir, H., 2007. Current Knowledge and Future challenges in Camelid Reproduction, Society of Reproduction and Fertility, vol. 64. Nottingham University Press, Nottingham, pp. 297–313.
32. Torner, H., Heleil, B., Alm, H., Ghoneim, I.M., Srsen, V., Kanitz, W., Tuchscherer, A., Fattouh, E., 2003. Changes in cumulus oocyte complexes of pregnant and nonpregnant camels (Camelus dromedarius) during maturation in vitro. Theriogenology, 60, 977-987.
33. Zeidan, A.E.B., Abd El-Salaam, A.M., El-Malky, O.M., Ahamdi, E.A.A., Sarhan, D.M.A., Daader, A.H., 2008. Biochemical and histological changes in the ovary of the dromedary camel during breeding and non-breeding seasons. Egyptian J Basic Appl Physiol, 7, 287 – 308.
34. Zeidan, A.E.B., El-Harairy, M.A., Gabr, Sh.A., Tag El-Dien, M.A., Abd El-Rahman, S.A., Amer, A.M., 2011. In Vitro Maturation of Camel Oocytes As Affected By Different Media during Breeding and Non-Breeding Seasons. Journal of American Science, 7(1), 460-472.
35. Zheng, W., Song, Y., Zi X., 2007. Effects of collection methods on recovery efficiency, maturation rate and subsequent embryonic developmental competence of oocytes in Holstein cows. Asian-Aust J Anim Sci, 20(4), 496–500.