Al-Muthanna J. For Agric Sci Print ISSN: 2226-4086 Online ISSN:2572-5149
Vol. 11 , Issue 2. 2024

https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/11.2/7

The effect of nitric oxide and the cumulus cells on the percentage of in vitro maturation (IVM) of buffalos normal ovaries oocytes.

Alaa Mohamed[1], Ali Al-HajObaid[2] and Muhammad Fakhrilden[3]

[1] Agriculture College, Al-Muthanna University, Iraq.

[2] Faculty of Medicine, Jabir Ibn Hayyan Medical University, Iraq.

Received on 01/9/2024 Accepted on 16/9/2024 Published on 15/12/2024

Abstract

This study was conducted in the laboratory of graduate studies in the Department of Animal Production / College of Agriculture University of AL-Muthanna and the laboratories of the of Jabir Ibn Hayyan Medical University during the period 22/9/2022 to 1/10/2023. The immature oocytes ware subjected to the experiment for in vitro maturation (UM) using the culture medium Dulbeccos Modified Eagles Medium (DMEM) treated with the nitric oxide with two concentrations: the low concentration was (3mM) and the high concentration was(5mM), to determine the effect of the cystic ovaries(follicular and luteal) ,the presence of cumulus oophorus and the concentration of nitric oxide in the percentage of (IVM) of buffalo oocytes. The result showed that the follicular cystic ovaries recorded the highest concentration of the insulin hormone(28.61± 0.15 U/mL) as compared with the luteal cystic ovaries (23.6 ± 0.29 U/mL) and the normal ovaries (24.41± 0.14 U/mL). On the other hand ,the normal ovaries recorded a highest concentration of LH (1.003 ± 0.028 U/ml) as compared with the follicular cystic ovaries (0.596 ± 0.02 IU/ml) and the luteal cystic ovaries (0.516 ± o.2 IU/ml). The luteal cystic ovaries showed the highest concentration of the prolactin hormone (1.050 ±0.03 ng/ml) as compared with the follicular cystic ovaries (0.883 ± 0.03 ng/ml) and the normal ovaries (0.600 ± 0.02 ng/ml) .

Key words: nitric oxide, cumulus cells, in vitro maturation (IVM), buffalos, ovaries oocytes.

Introduction

Since the beginning of the history in Iraq, The buffalo has been considered one of the animals whose existence was linked to the presence of the marshes, and they have become one of its distinctive signs for thousands of years. The archaeological drawings discovered depict us as the buffalo wrestling with a buffalo. With the passage of time, the Sumerians were able to domesticate this animal, which was once wild. Five thousand years ago, it was the companion and dearest friend of the people of the East in general and the Iraqis in particular [1].

The domestic water buffalo (Bubalus bubalis) is important in the agricultural economy of many developing countries in Asia, providing meat, milk and draft power. It is also used in some Latin American and Mediterranean countries as a source of meat and milk for local markets. [2].

Although buffalo can adapt to harsh environments and feed on poor quality fodder, reproductive efficiency is often compromised by these conditions, resulting in delayed sexual maturity, poor expression of estrus, prolonged postpartum estrus, and poor pregnancy rates and calving intervals. Age at puberty is affected by genotype, nutrition, management, and climate. Under ideal conditions, estrus occurs at 15-18 months in river buffalo and 21-24 months in swamp buffalo [3].

Polycystic ovary disease in buffaloes is generally divided into three types, including luteal fibrosis, which is fibrosis in the wall of the dominant follicles, or follicular cyst, which occurs in mature ovarian follicles. As for cysts in the tissue of the corpora lutea, then it is called cystic corpora lutea, and it is a non-pathological condition [4].

The current study aimed to demonstrate the effect of nitric oxide and the cumulus cells on the percentage of in vitro maturation (IVM) of buffalos normal ovaries oocytes.

Material and methods

Ovaries:

Two hundred and eighty buffaloes ovaries were evaluated from the types of polycystic ovary syndrome (PCOS) used. The source of buffalo eggs were ovaries obtained from the local Al-Muthanna slaughterhouse and the local Najaf slaughterhouse immediately after slaughtering the animals. The ovaries were diagnosed other, or presence of a follicular cyst, or presence of by a corpus luteum cyst.

Collection and classification of the buffalo ovaries and oocytes

Buffalo ovaries were collected from the local slaughterhouse in Muthanna and the local slaughterhouse in Najaf immediately after slaughtering the animals and placed in plastic containing a normal saline solution (0.9% sodium chloride) supplemented with antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin). Then it was placed. In a thermos at a temperature of 30-35 ° C. The ovaries were transferred to the laboratory of the College of Agriculture and the laboratory of the College of Medicine, Jabir Bin Hayyan Medical University, within less than 1-1.5 hours. In vitro, ovaries (panel 1) were washed three times with warm normal saline (37°C) described above to remove coagulation and reduce contamination on the surface of the ovaries [5].

The condition of the ovaries is diagnosed as normal or as having PCOS, which is classified according to the type of PCOS (cystic corpus luteum or cystic corpus luteum). Oocytes were collected from all follicles visible on the ovarian surface with a diameter greater than 2 mm using an aspiration technique [6].

Picture 1.buffalo ovaries after washing.

Oocyte Classified [7, 8].

***In vitro* Maturation (IVM)**

The oocytes were washed using SMART containing 5% HSA to remove substances in follicular fluid .The immature oocytes were directly distributed in average of 5 oocytes per droplet (0.5mL) from Dulbecco's modified Eagle,s medium(DMEM).

Results :

The result table (1) shows a non-significant differences between the treatments of nitric oxide concentration (low and high) and the presence of cumulus cells on the percentage of IVM of buffalo oocytes .

Table 1: The effect of nitric oxide and the cumulus cells on the percentage of in vitro maturation (IVM) of buffalos normal ovaries oocytes.

Treatment	No.	IVM (%)± standard error
control	20	4.32± 64.50
Low NO + CC	20	3.10± 68.50
High NO + CC	20	3.10± 67.00
Low NO. without CC	20	4.99± 66.00
High NO. without CC	20	3.73± 74.50
significant	---	NS
NS :Insignificant.		

low Nitric Oxide + cumulus , High Nitric Oxide + cumulus , low Nitric Oxide. without cumulus , High Nitric Oxide without cumulus

On the other hand thee result table (2) shows a highly-significant (P≤ 0.01) differences between the treatments of nitric oxide concentration (low and high) and the presence of cumulus cells on the percentage of IVM of buffalo oocytes. The treatment low concentration of nitric oxide records a highest percentage of IVM in the oocytes with cumulus cells was 74.50 ± 2.94 % and the lowest percentage was recorded in the control treatment (without nitric oxide) was 53.50± 1.31% and the treatment of high concentration of nitric oxide an cumulus cells was 53.50 ±3.64 %.

Table 2: The effect of nitric oxide and the cumulus cells on the percentage of in vitro maturation (IVM) of buffalos cystic ovaries oocytes.

Treatment	No.	IVM ± standard error %
Control	20	1.31± 53.50b
Low NO+ CC	20	2.94± 74.50a
High NO + CC	20	3.64± 53.50b
Low NO. without CC	20	4.16± 69.00a
High NO. without CC	20	2.10± 56.00b
significant	---	**
Averages with different letters within one column differ significantly from each other.**(P≤0.01).		

low Nitric Oxide + cumulus , High Nitric Oxide + cumulus , low Nitric Oxide. without cumulus , High Nitric Oxide without cumulus

Table (3) shows a significant (P≤ 0.05) differences between the normal & cystic ovaries in the control treatment in the percentage of laboratory maturation ,wherein the cystic ovaries records a low percentage of laboratory maturation 53.50± 1.31% as compared with the normal ovaries 64.50± 4.32%, while treatments of low concentrations of nitric oxide records a non-significant differences in the percentage of laboratory maturation both in case with presence or non-presence of cumulus cells whereas the treatment of high concentration of nitric oxide records a highly-significant (P≤0.01) differences between the normal & cystic ovaries in the percentage of laboratory maturation wherein the normal ovaries records a highly percentage of laboratory maturation 67.00 ±3.11% as compared with the cystic ovaries 53.50± 3.64% with presence of cumulus cells and the normal ovaries records a highly percentage of laboratory maturation 74.50 ±3.73% as compared with the cystic ovaries 56.00± 2.10% without the cumulus cells.

Table 3: The correlation between the normal & cystic ovaries in the percentage of laboratory maturation

Treatment	IVM ± standard error %		Sig.
	Natural ovaries	Polycystic ovaries	
control	4.32± 64.50	1.31± 53.50	*
Low NO + CC	3.10± 68.50	2.94± 74.50	NS
High NO + CC	3.10± 67.00	3.64± 53.50	**
Low NO .without CC	4.99± 66.00	4.16± 69.00	NS
High NO .without CC	3.73± 74.50	2.10± 56.00	**
(*P≤0.05) ** ،(P≤0.01).			

low Nitric Oxide + cumulus , High Nitric Oxide + cumulus , low Nitric Oxide. without cumulus , High Nitric Oxide without cumulus

Discussion:

The reproductive efficiency of buffalo is relatively poor regardless of their location throughout the world. Buffalo exhibit several well-known reproductive problems including delayed onset of puberty, poor onset of estrus, ovarian inactivity for a longer period after birth, and most importantly, lower pregnancy rates, especially when raised in pens [9].The buffalo fertility can be achieved by improving management and nutrition [10]. The reproductive fitness of buffalo species is determined by many different processes, which result from the interaction between genetics and environment, it includes the age of puberty or maturity, the form of the estrus period and the behavior during the estrus, the rate of ovulation, the lactation period, the period of postpartum estrus, the period between births, and the duration of reproductive life, a combination of these traits is used to characterize breeding efficiency in farm animals [11].

The polycystic ovarian syndrome (PCOS) in general is a dysfunction of the ovaries of livestock, which causes reproductive failure, prolonging the period between two births, and raising production costs, which led to major economic losses for livestock breeders. It is characterized by the presence of permanent follicular or luteinized structures on the ovaries [12, 13].

The hormonal disorders causing polycystic ovary syndrome. Although there is a wide range of studies that have addressed the biological mechanisms of polycystic ovary syndrome, the exact causes of its occurrence are still unknown [14].

References:

[1] Youssef, K., S.M. Sanzani, A. Ligorio, A. Ippolito and L.A. Terryc (2014). Sodium carbonate and bicarbonate treatments induce resistance to postharvest green mould on citrus fruit. Postharvest Biology and Technology, 87: 61-69.

[2] Gautam, G., Gyawali R. R, Nath, B. and Pant, S. (2017). Characterization and treatment of infertility in buffaloes of hills and Terai regions of mid Nepal. Proceedings of the International Buffalo Symposium, 15-18 Nov 2017, Chitwan, Nepal; pp. 98-102.

[3] Wanapat M. and P. Rowlinson (2007). Nutrition and feeding of swamp buffalo: feed resources and rumen approach. Ital. J. Anim. Sci., 6 (Suppl. 2): 67-73.

[4] Teshome, E., Kebede, A., Abdela, N. and Ahmed, W. M. (2016). Ovarian cyst and its economic impact in dairy farms: A review. Global Veterinaria, 16, 461–471.

[5] Rizk, B. (2009). Symposium: Update on prediction and management of OHSS. Genetics of ovarian hyperstimulation syndrome. Reprod Biomed Online 19(1):14-27

[6] DeSmedt V., N. Crozed, M. Ahmed-Ali, A. Martino and Y. Cogine (1992). In vitro maturation and fertilization of goat cells. Theriogenology 37: 1049-1060.

[7] Nogueira D., R. Ron-El, S. Friedler, M. Schachter, A. Raziel, R. Cortvrindt and J. Smitz (2009). Meiotic arrest in vitro by phosphodiesterase 3-inhibitor enhances the maturation capacity of human oocytes and allows subsequent embryonic development. Biol. Reprod., 74:177–184.

[8] Cavilla, J.L., C.R. Kennedy, A.G. Byskov and G.M. Hartshorne (2008). Human immature oocytes grow during culture for IVM Hum Reprod, 23: 37-45.

[9] Gordon, L., (2002). Oestrus and the oestrus cycle in cattle. in: Controlled Reproduction in cattle and buffaloes. 2th Edition. C. A. B. international company. USA. pp: 123-124.

[10] Qureshi, MS, Khan S, Ahmad N, 2007. Pregnancy depresses milk yield in dairy buffaloes. Italian J Anim Sci. ;6(Suppl 2):1290–1293.

[11] Agrawal, K.P., 2003. Augmentation of reproduction in buffaloes. Proceedings of 4th Asian Buffalo Agric. Ricerca. 153:73-78.

[12] Vanholder, T., Opsomer, G. & De Kruif, A. (2006). Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reproduction Nutrition Development. 46(2), 105–119. DOI: https://doi. org/10. 1051/rnd:2006003.

[13] Rose. W. J (2012). Diaynosis and mechanisms of bovine ovarian cysts. phD thesis, University of Nottingham.

14] Peter AT (2004). An update on cystic ovarian degeneration in cattle. Reprod. Domest. Anim. 39: 1-7.