Research Article

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Applying Modified 2000 *In Vitro* Technique for Maturation of *B. Indicus* Oocytes by Using Plastic Test Tubes Supplemented with M-Pbs Medium

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Abstract

This project was conducted in Health and Medical Technical College's laboratories of Al-Forat Al-Awsat Technical University in 2021. The study was dealt 370 oocytes collected from 19 three years old cows by using dissecting process. The gathered oocytes were transferred hastily to a test tube contains m-PBS prelude for incubation by modified *in vitro* technique 2000, which supplemented with 5% Co_2 gas state for 26 hours at 37°c. The gained consequences led to 20% maturation occurred for the second metaphase after applying m-PBS and staining with orcein stain. Statistics supports our hypothesis.The outcomes for this study is that incubation of *B. indicus* oocytes *in vitro* by modified technique 2000 with using m-PBS culture media will established a success and promising in maturation rates.

Keywords: *B. indicus*, oocytes, *in-vitro* maturation, Co_2 gas stage, m-PBS.

Introduction:

In vitro techniques as well as embryo transfer methods offered an active lab, technical and scientific procedures used for treatment problems of sterility and reproductive dysfunctions or disorders (1). IVF and handling both sperms and oocytes in the laboratory aid effectively in application of embryo transfer techniques to overcome reproductive problems (2).

Specific anatomical and physiological studies gametes introduce benefits for progression in this line of medical science. These studies included immunological side, capacitation, and later, embryo freezing, that paving up the way for understanding molecular, cytological and physiological processes all fulfillments of *in vitro* fertilization experiments in all animals as well as human (3).

The maturation *in vitro* must be mimic the same phenomenon conditions as *in vivo,* So, the in vivo trip crossing up through stages of primordial follicle growth, folliculogenesis, oocyte growth, atresia, and at last oocyte maturation. On the other hand, *in vitro* maturation begins after collecting semi-ovulated oocytes from the donors before introducing them to the incubation systems over there (4).

Aims of this study were relying on *in vitro* maturation of bovine oocytes in PBS medium through using modified culture technique 2000.

Materials and method:

Site of action: This procedure was performed in Health and Medical Technical College's laboratories of Al-Forat Al-Awsat Technical University in Kufa- march/2021.

Animal model: three years old local cows (*Bos indicus*) were used in this study. In the abattoir, they were slaughter to collecting their ovaries, placed in ice bag, and then transported to the laboratory.

Oocytes collection: 370 oocytes were collected surgically by scalpel in the lab, then divided into two group before transferring to culture medium.

Culture medium m-PBS: it was purchased from commercial scientific store through. It was manufactured by Biocompare, Inc.- USA.

Maturation *in vitro* technique: 10 oocytes were put in one test tube contain 2.5 ml of m-PBS. Each test tube plugged with a rubber stopper with two short tubes, one for Co2 gas inlet that's coming from a bottle gas, and another one was for gases outlet. All test tubes fixed in shaking water bath with a 37oc for 28 hr. (5).

Investigation: matured oocytes had been fixed with absolute methanol/glacial acetic acid for 24 hr. in a preparation way for staining with orcein before investigated at compound microscope.

Statistics: T-student test was applied for confirming the confident probability (6).

Results

Data showed a 20% (74) of dealt oocytes was matured to the second metaphase stage.

To reaching this stage occurrence was through using each of test tubes as containers, 5% Co2 gas stage, just two determined degrees for cultured oocytes, 28 hours of maturation time *in vitro*, a 3 years old cows, and m-PBS culture medium. The stages of model's oocytes nuclear maturation were recorded and tabulated in table-1 below. It was clear that first phase was seen at the first hour of cultivation was GVBD with a percentage 11%. While the last phase metaphase II was looked at 28 hours after culturing, with a percentage 92%.

In the same line, its looked that first appearances of metaphase I happened after 8 hours of incubation. It looks like a matter correlated with incubation's time progression that most of oocytes scored metaphase II stage after 20 hours of incubation in this research.

Images-1 and -2 below showed the metaphase I and metaphase II of an oocytes incubated for 16-20 and 24-28 hours respectively.

Table-1: Phases of nuclear development and divisions in oocytes incubated from 1 minute to 28 hours by test tubes method 2000.*

Phases of oocyte's nuclear development								
Metaphase II	**Telophase I**	**Anaphase I**	**Metaphase I**	**Diakinesis**	**Germinal vesicle breakdown**	**Germinal vesicle**	**Number of oocytes**	**Culture period**
-	-	-	-	-	5 (11%)	40	45	1
-	-	-	-	5	35 (78%)	5	45	4
-	-	-	5	30 (67%)	10	-	45	8
-	2 (5%)	3 (6%)	20	20	-	-	45	12
-	4 (9%)	12 (27%)	28 (62%)	1 (2%)	-	-	45	16
1 (2%)	9	22 (49%)	13 (29%)	-	-	-	45	20
22 (49%)	12 (27%)	6 (13%)	5 (11%)	-	-	-	45	24
51** (93%)	3 (5%)	1 (2%)	-	-	-	-	55	28

***The dependent statistical illustration equation is percentage %.**

***T-student test is the main statistical test used for hypothesis testing.**

****Statistically significant difference; p> 0.05.**

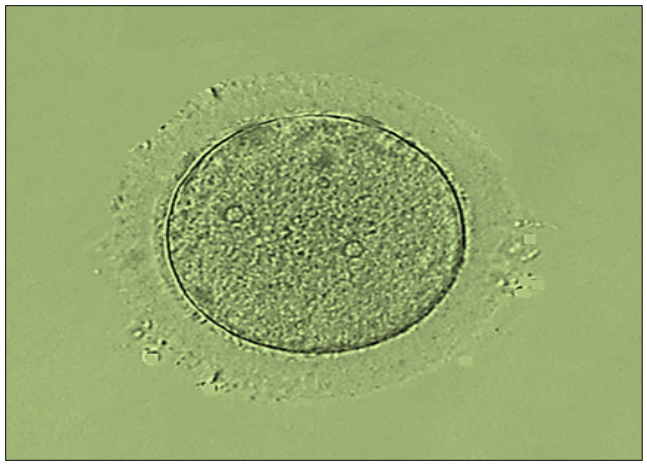

Image-1: *In vitro* matured cow's oocyte in stage metaphase I with size 148 µm (30× magnification).

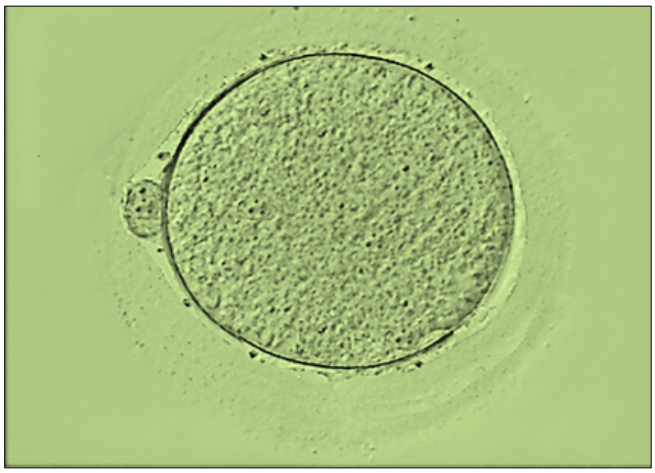

Image-1: *In vitro* matured cow's oocyte in stage metaphase I with size 148 µm (30× magnification).

Discussion

The dissection method for collecting oocytes for *in-vitro* maturation assist perfectly in getting more cumulated oocytes in grade-1

and grade -2 with a percentage (70%), as a best choice in this research in comparison with the same and other methods applied previously (7).

Culturing system of test tubes confirmed the efficiency of growth most of oocytes to the metaphase II stage with a percentage (60%), especially when it had applied within the modified 2000 culturing system had a good thermal and vibration homogeneity and good air exchange when it is compared with other systems applied by (5, 8, 9).

The stages of oocytes nuclear maturation in this study gave no significant differences from the original one, in spite of the slight decline in metaphase II appearances in the past studies. So, the present study showed that GVBD stage had occurred after 4 hours of cultivation *in-vitro,* in a time that metaphase II begun to formed after 20 hours of maturation. These results concurred with previous studies in the same field of IVM (10)(11).

5% Co2 gas phase had applied by many studies to mimic the internal environment conditions inside the female reproductive system. In this experiment no obvious significant differences from the previous results obtained by the original study in the levels of maturation rates. This gas phase kept the continuity of the culture medium permeability to prevent the acidity occurrence that disturbing the maturation activity. Our results compatible with those of (12) and (13).

The using of m-PBS culture medium in our study gave a good choice for continuity of maturation to the premium phases of growth. our study promoted (70%) GVBD occurrence cases if it was compared with other studies of (14, 15).

the oocytes those reached the metaphase II were being higher by using m-PBS medium in agreement with the original studies for IVM tests. So, in this work the oocytes in metaphase ii recorded a 20% maturation percentage after 28 hours of incubation (16, 17).

On the other hand, the incubation temperature at 370c by using both of test tubes and m-PBS, had accomplished these significant results as it is mentioned previously (5).

The modified PBS medium reflects its nutritional values and efficiencies assisted in the growing and maturation of cow's oocytes cultivated in it especially NaCl, KCl, Na2HPo4, KH2Po4, and glucose (18, 19).

Test tubes types as a plastic in this study put a premium priority for their unbreakable manufactured nature and well suited with common reactive chemicals in the laboratories under control tasks (20).

The main conclusions of our present study is for maturating cow's oocytes *in-vitro* perfectly to the acceptable limits by using modified IVM 2000 method that based on using shaker water bath, 5% Co2, plastic test tubes, and m-PBS medium. All these factors were giving rise to a good choice for maturation *in vitro* in compared with other studies.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تطبيق تقنية 2000المعدلة في المختبر لإنضاج بويضات ابقار *B. indicus* باستخدام انابيب اختبار بلاستيكية مدعمة بوسط m-PBS

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الخلاصة

تم تنفيذ هذا المشروع في مختبرات الكلية التقنية الصحية والطبية بجامعة الفرات الأوسط التقنية في عام .2021تم التعامل مع الدراسة 370بويضة تم جمعها من 19بقرة عمرها ثلاث سنوات باستخدام عملية التشريح .تم نقل البويضات المجمعة على عجل إلى أنابيب اختبار تحتوي على مقدمة m-PBS للحضانة بواسطة تقنية معدلة في المختبر 2000، والتي استكملت بحالة غاز CO2 بنسبة 5٪ لمدة 26ساعة عند 37درجة مئوية. أدت العواقب المكتسبة إلى نضوج 20٪ حدث للمرحلة الاستوائية الثانية بعد تطبيق m-PBS وتلطيخ صبغة الأورسين .الإحصاءات تدعم فرضيتنا .نتائج هذه الدراسة هي أن حضانة البويضات B. indicus في المختبر بواسطة تقنية معدلة 2000باستخدام وسائط ثقافة m-PBS ستحقق نجاحا وواعدة في معدلات النضج .

الكلمات المفتاحية: *B. indicus*، البويضات، النضج في المختبر، مرحلة غازCO2 ،.m-PBS .