



## EFFECT OF USING DIFFERENT OVARIES TRANSPORTATION SOLUTIONS IN OVARIAN FOLLICLES AND OOCYTES VIABILITY AND ABNORMALITY IN OVINE

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### Abstract

The present study objective is to study the using different solutions effect when transport the ovine ovaries from the slaughter to the Lab on the abnormalities and mortality percentages of ovarian follicles and oocytes. In this work, ewes that were slaughtered in Ghammas slaughter were used. This work was conducted in the College of Agriculture-University of Al-Qadisiyah from October 2018 to March 2019 and has been collected 100 ovine ovaries, this study consists of one experiment shows the impact of the use of solutions to transport ovaries from the slaughter to the Lab (salt solution, phosphate solution and sugar glucose solution) On the proportion of vitality and abnormalities in the oocytes and ovarian follicles. The oocytes were aspirated and the ovarian follicles were isolated prior to the formation of follicle fluid (primary, secondary and tertiary) from the ovaries and were tested for vitality and calculation of the percentage of Ovarian follicles and oocytes using trypan blue dye. The results of this work noted a significantly ( $P < 0.05$ ) decline in the Ovarian follicles and oocytes abnormalities percentage when the ovaries transport with phosphate solutions and glucose solution compering with physiological saline solution. In the other hand there were non-significant different between work groups in the results of abnormality percentage of (primary, secondary and tertiary) ovarian follicles. While significantly decreased ( $P < 0.05$ ) was observed in antral follicles when used glucose solution. In conclusion he ovarian transport was better when used phosphate solutions and glucose solution.

### Introduction

When mammals are born, there are many ovarian follicles in the ovary. However, the huge superiority of these follicles do not reach ovulation, but expire during advancement and maturation and turn into atresia follicles, several oocytes are produced in the female reproductive age (Carroll *et al.*, 1990). In recent years, many studies were focused on many follicles of the ovaries. Different protocols were developed for the oocytes and follicles isolation and *in vitro* maturation in bovine ovaries (Gutierrez *et al.*, 2000), buffaloes (Abd-Allah, 2009; Abd-Allah, 2010).

New biotechnologies develop the follicles (pre-antral fluid) in the ovaries before they become atrophic and mature *in vitro* until they reach maturity (Figueiredo *et al.*, 2000). The quality of the follicles before the formation of the follicle fluid after during the transport and removal of the ovaries, since the donor ovary of the follicle follicles before the formation of follicle fluid in Lab studies is usually far from reproductive laboratories. During transport to the Lab is important when using pre-antral follicles in cryopreservation protocols and / or *in vitro* maturation (Figueiredo *et al.*, 2000; Ibrahim *et al.*, 2019).

Different solutions were used in ovarian transport. A 0.9% saline solution was used to transport ovaries from cows (Scherthaner *et al.*, 1997), ovine (Westhof *et al.*, 1991). Phosphates are largely in the transport of metabolites from ovine (Smith *et al.*, 1994.), cows (Niemann, 1991), mice (Nureddin *et al.*, 1990), rabbits and cats (Jewgenow *et al.*, 1998).

Love *et al.* (2003) have indicated that the division rate of mature oocytes is high in phosphate-containing media at 38 °C. In contrast to these findings (Evecen *et al.*, 2010; Al-Bachry *et al.*, 2019) show that the percentage of division of mature oocytes has decreased in phosphate-containing media. These discrepancies may be due to varying length of storage

time. Enzymes, hormones and pH in the physiological range. The absence of a pH regulator when using a physiological salt solution resulted in a decrease in pH, which significantly increased DNA methylation. The oocytes plasma membrane has high permeability of H<sup>+</sup> ions even regulatory mechanics (Wongsrikeao *et al.*, 2005). If the external cells pH is fixed the oocytes by increasing the buffer solutions, the protons will be excreted, which leads to a decrease in the pH inside the cells (Salehi *et al.*, 2004.), and this is considered to be of importance of biological in regulating the net electrical charge of the amino acids, which leads to a change in the permeability of the plasma membrane. (Patel *et al.*, 1973.). For oocytes ripening, energy metabolism is essential as it takes a lot of energy to advance all the powerful preserver complex. It need an accident of energy from various origin contain carbohydrates and amino acids (Collado-Fernandez *et al.*, 2012.). Several studies have shown the beneficial effect of glucose metabolism on the maturity of the oocyte (Sutton-McDowall *et al.*, 2010). Whereas, the high level of diabetic degradation is associated with the resumption of meiosis in the oocytes (Steeves and Gardner, 1999; Cetica *et al.*, 2002).

The objective of this work is to study the effect of using different solutions to transport ovaries from the to the Lab on the death rate, oocytes abnormalities, and ovarian follicles in ovine.

### Materials and Methods

This work was carried out using ewes ovens that were slaughtered in the Ghamas massacre, west of Diwaniyah. This work was conducted in the College of Agriculture - University of Al-Qadisiyah from October 2018 to March 2019, and 100 ovaries were collected from ovine, 70 of which were distributed randomly to the study groups, then the oocyte were withdrawn from the ovarian optic ovaries, the three follicles 2-10 mm, and then isolated the follicles of

the ovaries. The remaining ovaries (32 ovaries) were divided randomly to the groups as well, and then were used for the purpose of Chopping the tissues of the ovarian cortex.

### Study design

This work consisted of one experiment showing the effect of the use of different solutions (local physiological solution, phosphate solution and glucose solution) during the transport of ovaries from the slaughter to the Lab on the rate of deformities and mortality in the follicles of ovaries and oocytes.

### Transportation solutions preparation

#### Normal saline solution

The physiological saline solution made by dissolved 8.5 g NaCl salt in 1 liter of distilled water with the antibiotics addition (100 g / ml streptomycin, 100 g / ml metronidazole and 100 IU / ml penicillin).

#### Phosphate solution

A Phosphate solution was made by dissolved 5 g of  $\text{NaPO}_3$  with 0.5 g of aqueous potassium phosphate ( $\text{KHPO}_3$ ) in 100 ml distilled water (Miyoshi, 2016.) with the antibiotics (100 g / ml streptomycin, 100 g / ml metronidazole and 100 IU / ml penicillin).

#### Glucose solution 5%

The preparation of Glucose solution 5% was by dissolved 5 g of glucose sugar in 100 ml of distilled water (Dextrose, 2017) with the antibiotics addition (100 g/ml streptomycin, 100 g/ml metronidazole and 100 IU/ml penicillin).

### Collection of ovaries

Ovine ovaries collected from a slaughter (Ghammas) in Diwanayah governorate. Immediately after slaughter, the ovaries of each animal were collected and placed in plastic containers were contained the transport solution of the treatment, and placed in a thermally insulated container (thermos) at 30 °C. The ovaries were transported to the Lab in the College of Agriculture, University of Al-Qadisiyah, within four hours. In Lab, washed ovaries for three turns with the transport solution of the treatment. This process removes blood clotting and reduces contamination on the surfaces of the ovaries (Rezk, 2009).

### Oocytes collection

Oocytes were collected from large follicles on the surface of the ovary (2-7) mm using the technique of aspiration using disposable syringe 5 ml carrying a 20-gauge syringe containing 1 ml of the transport solution for the treatment. After the oocytes were withdrawn, each of syringe containing the oocytes placed to petri dish and by dissecting microscope, then collected the oocytes by a micro pipette and there were washed three turn with the transport solution (DeSmedt *et al.*, 1992).

### Pre-antral follicles Isolation

After oocytes aspirated from the large ovarian follicles, the ovary is cut into two halves. Then the ovarian cortex was removed from both halves, then the ovarian cortex was cut by an anatomical scalpel, and then using a microscope it was carried out by the Pasteur Pipette. The vesicles are then released continuously to rid them of the remaining tissue and

then the follicles were washed. With group transport solution (Patricia *et al.*, 2009).

### Preparation of Trypan blue dye

A 0,4% blue trypan tincture solution prepared by dissolving of trypan dye powder 0.4 g in phosphate buffer solution 100 ml and then filtered using a filter paper. It was placed in a dark glass container and then kept in the refrigerator (Fakhrildin and Al-Moussawi, 2013).

### Viability test

Examination of the vitality of all primary, secondary and tertiary ovarian follicles by placing a drop of the trypan blue stain solution to the ovules and ovarian follicles. Then the results were recorded as the cells that did not stain the cytoplasm are considered viable and vice versa in which the cytoplasm is stained meaning that they are mortal (Abd-Allah, 2010; Al-Tawash *et al.*, 2020).

### Ovarian cortex fragments: preparation

The cortex of each ovary was divided into small pieces of size 2\*4\*1 mm and there were placed to tubes contained formalin 10% for histological sections (Allen and Cameron, 2004)

### Statistical analysis

statistical analyze of data was made using the completely randomized design (CRD), and the differences of means between the groups were measured using Duncan's Multiple Range test (Duncan, 1955).

## Results and Discussion

### Effect of ovarian transportation solutions on the percentage of mortality of oocytes:

The results of effect of transport of ovarian from the slaughter to the Lab on the rate of oocytes abnormalities showed a significantly ( $P < 0.05$ ) increase in the mortality of oocytes when transporting ovaries using physiological solution where (27.5%) compared to the use of phosphate and glucose solutions (21.7%) (figure 1). This is consistent with the results of the use of the physiological salt solution in the reduction of pH, which significantly increased the DNA titration, although The oocytes plasma membrane has high permeability of  $\text{H}^+$  ions even regulatory mechanics (Wongsrikeao *et al.*, 2005). If the external cells pH is fixed the oocytes by increasing the buffer solutions, the protons will be excreted, which leads to a decrease in the pH inside the cells (Salehi *et al.*, 2004), and this is considered to be of importance of biological in regulating the net electrical charge of the amino acids, which leads to a change in the permeability of the plasma membrane (Patel *et al.*, 1973)

### Effect of ovarian transport solutions on the percentage of oocytes abnormality:

Figure (2) shows the results of the effect of ovarian transport solutions on the rate of oocytes abnormalities significantly ( $P < 0.05$ ) decline in the percentage of oocytes abnormalities when transporting ovaries using physiological solution where (32.5%) compared with the use of phosphate and glucose solutions. Bohlooli *et al.* (2015) contend that degradation of glucose is the main anaerobic pathway for ATP production, which should lead to increased lactate levels and low pH under hypoxic conditions. Cell membrane permeability and cell membrane charge (Patel *et al.*, 1973.)

and instability of genetic material in oocytes and ovarian follicles.

**Effect of ovarian transport solutions on percentage of abnormality of Primary, secondary and tertiary ovarian follicles**

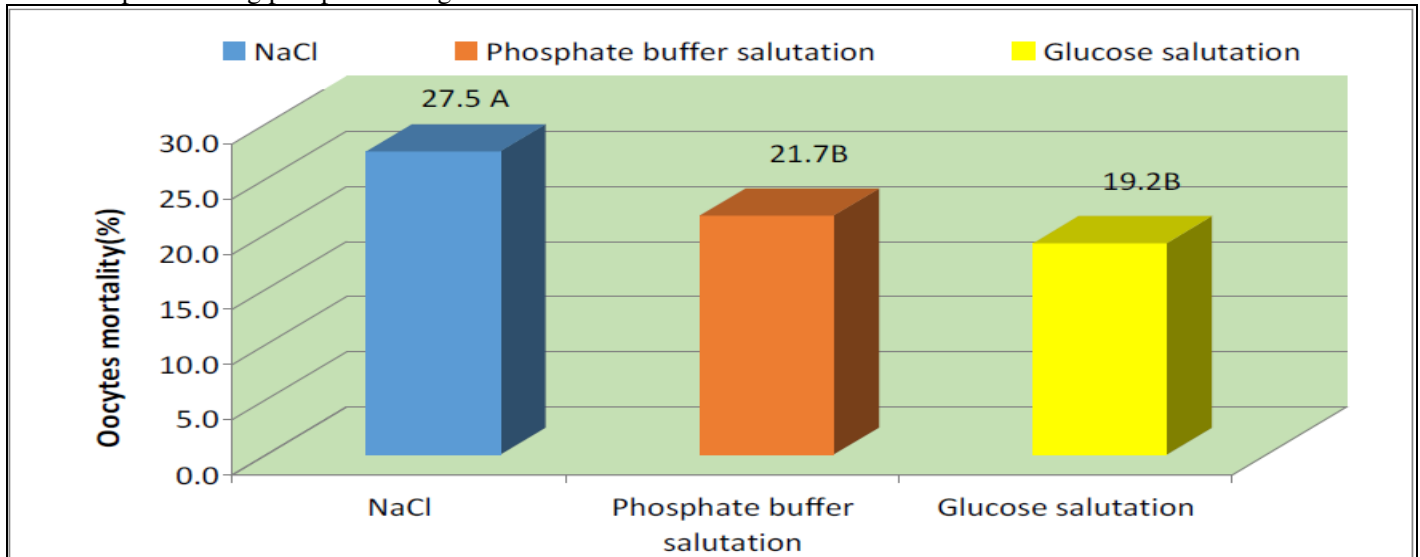
Studies conduct that grape glucose in the presence of phosphate is trustworthy for delaying or cramp consequence in peach-tier embryos in transplantation media (Thompson *et al.*, 1992). Increased phosphate in the average did not reprove embryo development. In oppose, the complete elimination of phosphate has amended developmental efficiency (Schini and Bavister, 1988). However, it is not understood whether phosphates in ovarian transport solutions have an indirect or positive effect on oocytes developmental and maturation efficiency. On the other workmanship, between Lopes *et al.* (Lopes *et al.*, 2009) these results contradict the results of this study (figures 3,4 and 5 respectively) where the use of different solutions to transport ovaries from the slaughter to the Lab on the proportion of Primary, secondary and tertiary ovarian follicles significantly ( $P < 0.05$ ) decrease (in Primary, secondary and tertiary The ovarian follicles when ovaries were transported using phosphate and glucose solutions were

recorded (33.6%) and (33.8%), respectively, while they recorded a significant ( $P < 0.05$ ) increase when using the physiological solution (36.0%).

**Effect of ovarian transport solutions on the percentage of abnormalities of the antral ovarian follicles**

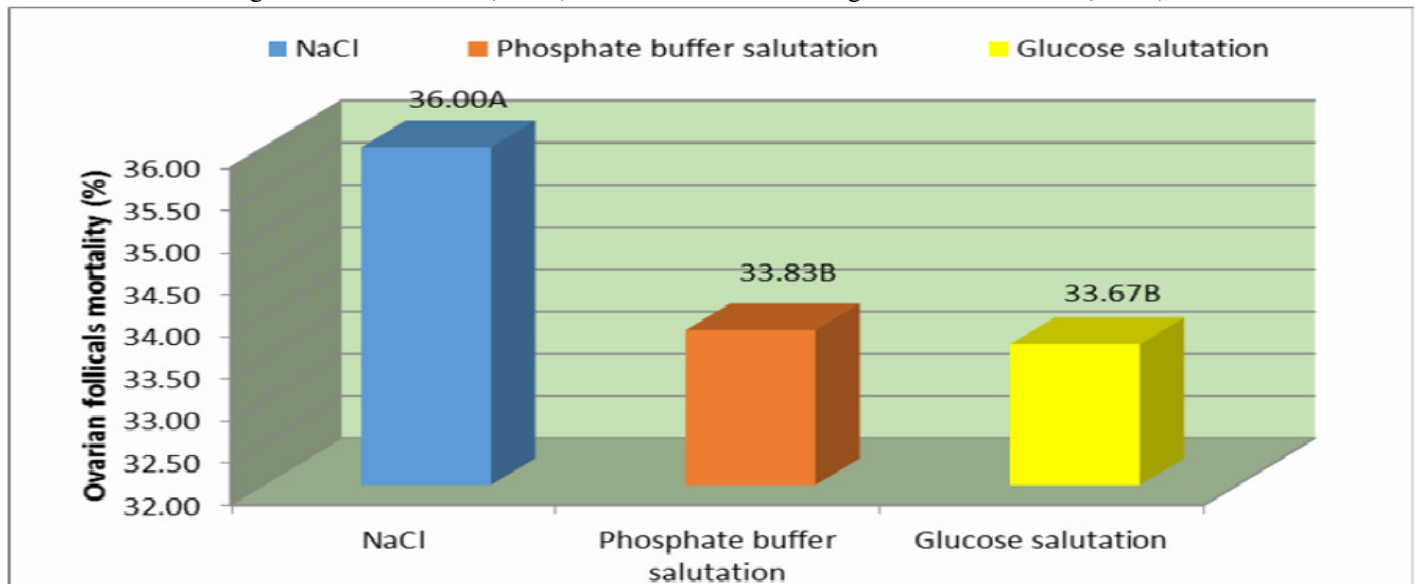
The results of the effect of the transport of ovaries from the slaughter to the Lab on the rate of abnormalities of the antral ovarian follicles significantly ( $P < 0.05$ ) in the transport of ovaries using glucose solution (20.6%) compared with the use of phosphate and physiological solutions (24.8%) (Figure 6). These results were consistent with the results of Love *et al.* (2003) where the rate of division of mature oocytes was high in phosphate-containing media at 38 °C. Evecen *et al.* (2010) reported that the rate of division of mature oocytes has decreased in circles containing phosphate may be these differences date back to the different length of time of storage, the use of phosphate in the media used in the transport of the ovaries led to the preservation of enzymes and hormones and pH in the physiological range.

In conclusion the ovarian transport was better when used phosphate solutions and glucose solution.



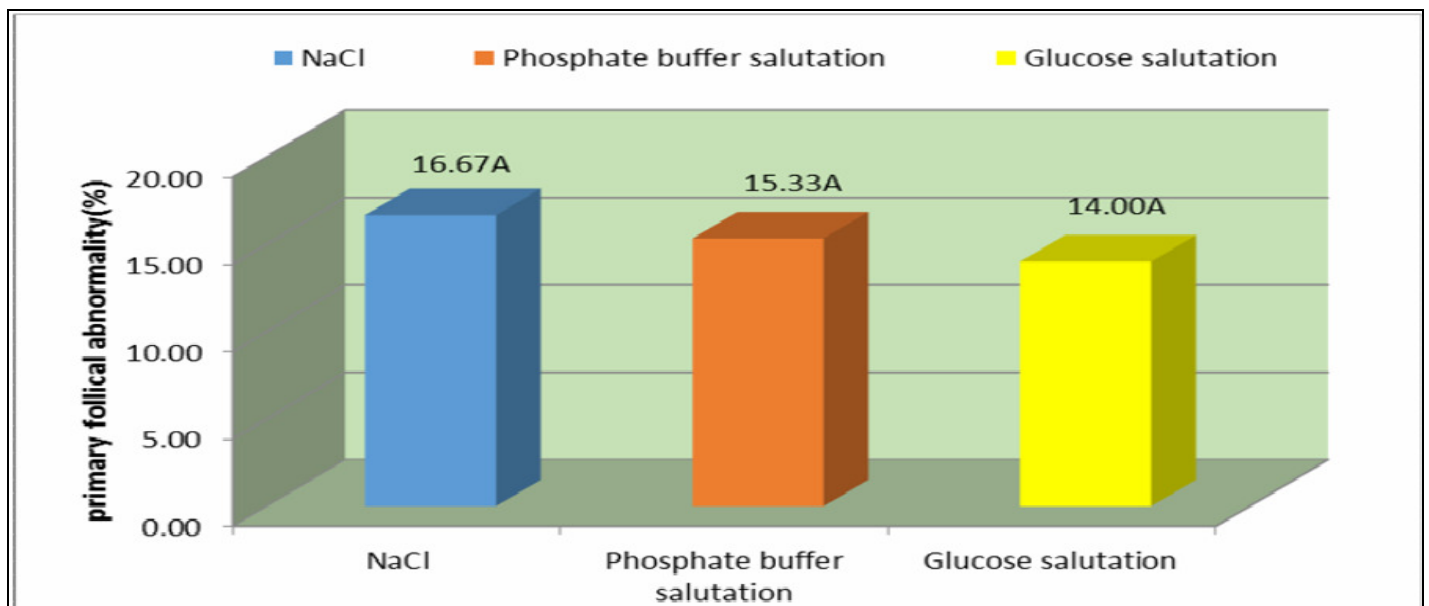
**Fig. 1 :** Effect of transport solution on oocytes mortality percentage

• Simi letters are non-significant deferments ( $P \geq 0.5$ ) Different letters are significant deferments ( $P < 0.5$ )



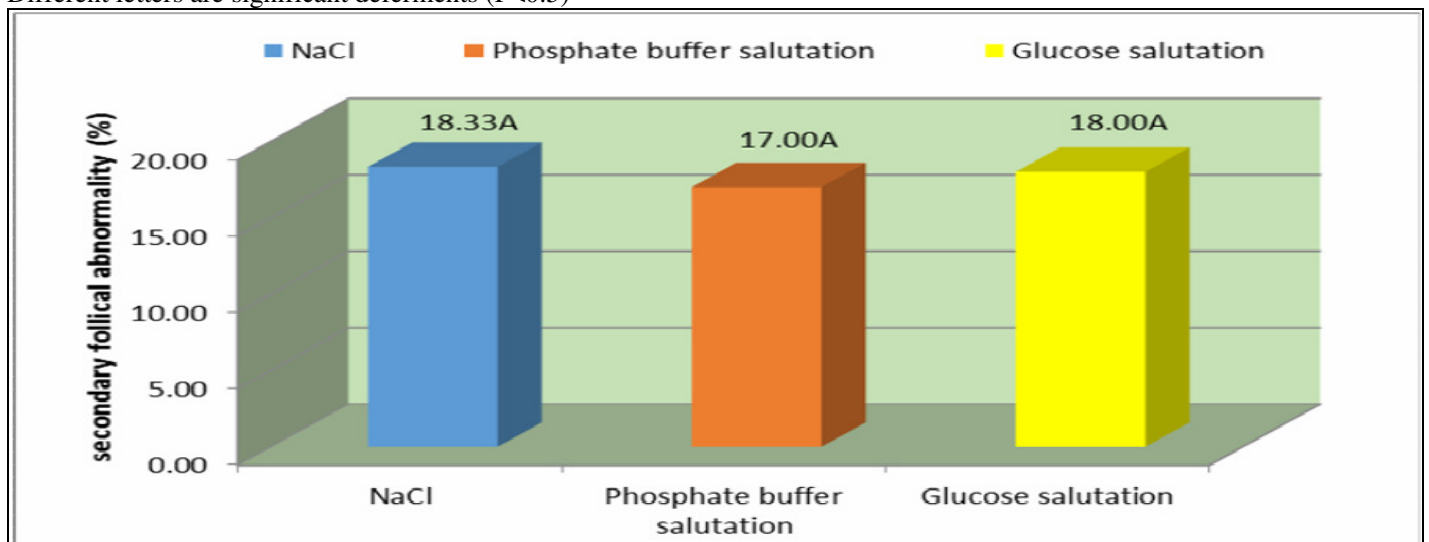
**Fig. 2 :** Effect of transport solution on ovarian follicle mortality percentage

• Simi letters are non-significant deferments ( $P \geq 0.5$ )  
 • Different letters are significant deferments ( $P < 0.5$ )



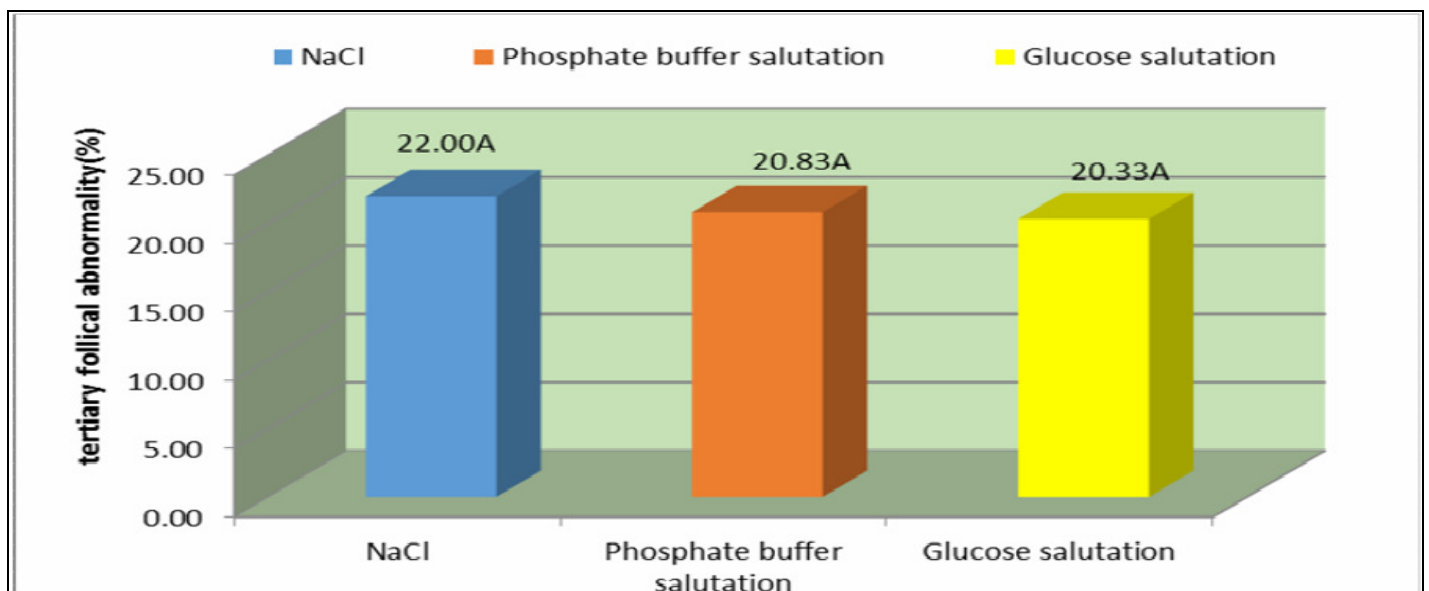
**Fig. 3 :** Effect of transport solution on primary ovarian follicle abnormality percentage

- Simi letters are non-significant deferments ( $P \geq 0.5$ )
- Different letters are significant deferments ( $P < 0.5$ )



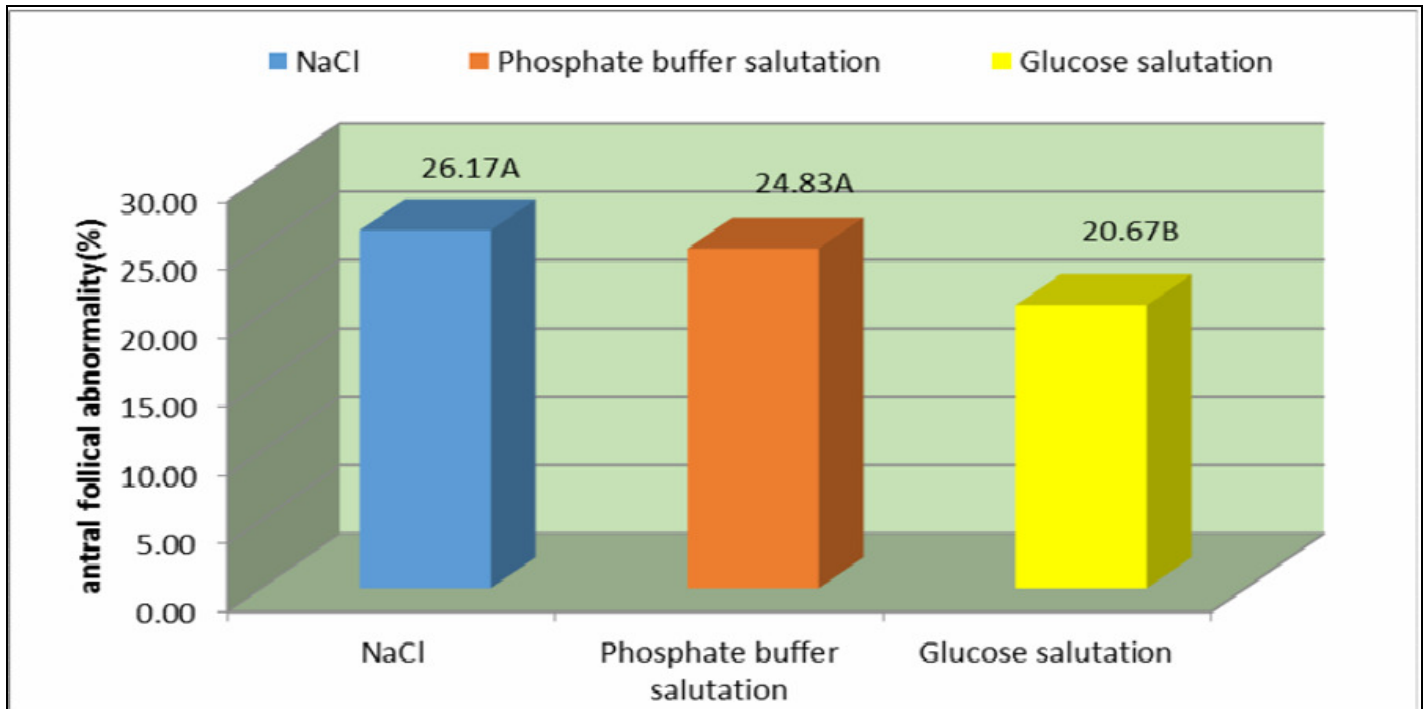
**Fig. 4 :** Effect of transport solution on secondary ovarian follicle abnormality percentage

- Simi letters are non-significant deferments ( $P \geq 0.5$ )
- Different letters are significant deferments ( $P < 0.5$ )



**Fig. 5 :** Effect of transport solution on tertiary ovarian follicle abnormality percentage

- Simi letters are non-significant deferments ( $P \geq 0.5$ )
- Different letters are significant deferments ( $P < 0.5$ )



**Fig. 6 :** Effect of transport solution on antral ovarian follicle abnormality percentage

- Simi letters are non-significant deferments ( $P \geq 0.5$ )
- Different letters are significant deferments ( $P < 0.5$ )

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