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*Article*

# Effect of Gonadotropin Hormones on Viability, Morphology and In Vitro Maturation of Sheep Oocytes

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**Abstract:** The study investigates the impact of gonadotropin hormones on the viability, morphology, and in vitro maturation of sheep oocytes, addressing a knowledge gap in optimizing culture conditions for oocyte maturation. Using a controlled experiment, immature sheep oocytes were divided into groups with varying concentrations of hCG and PMSG hormones and incubated under specific conditions. The findings revealed that the addition of gonadotropins significantly increased oocyte viability, with the highest viability and normal morphology observed in the group with the highest hormone concentration. These results underscore the critical role of gonadotropins in enhancing oocyte maturation, with implications for improving in vitro fertilization techniques in mammals.

**Keywords:** In Vitro Maturation (IVM), Gonadotropin, FSH, Immature Oocyte, Sheep, Hcg.

## 1. Introduction

In vitro maturation (IVM) is a crucial process in enhancing the maturity of oocytes in a laboratory setting. Through the process of oocyte maturation In vitro, the oocytes acquired significant developmental potential gradually until the embryogenetic activation following fertilisation (1,2). The concept of in vitro maturation of immature oocytes (IVM) was initially elucidated by Pincus and Enzmann in 1935 (3). Using immature oocytes derived from antral follicles, Cha and his colleagues recently achieved successful use of the IVM approach in humans (4).

To achieve complete maturation of oocytes, both cytoplasmic and nuclear maturation must take place efficiently. Nuclear maturation is exemplified by the reversal of the initial meiotic arrest during the germinal vesicle stage (GV) to the subsequent meiotic arrest during metaphase II stage (MII) (5). Cytoplasmic maturation encompasses several processes such as the storage of RNA and proteins, structural changes including the redistribution and distribution of cortical granules, migration of mitochondria to a perinuclear position, and the establishment of the calcium control system (6).

In vivo, the gonadotropin hormones are crucial for regulating the maturation and development of oocytes. Undoubtedly, the impact of gonadotropin relies on its crucial physiological function in facilitating communication between oocyte cumulus cells. It is highly beneficial for the development of the cytoplasmic and nuclear maturation of the cumulus oocyte complex (7).

The optimisation of the culture system for oocyte maturation involves considering the key factors necessary for the effective in vitro generation of embryos in mammals. To enhance the culture conditions, it is recommended to include gonadotropin hormones,

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such as follicle-stimulating hormone (FSH), into the medium. FSH has the ability to enhance the mRNA expression of gonadotropin receptors in cumulus cells, therefore ensuring their growth and ultimately leading to the maturation of oocytes (8,9).

In vitro maturation of mammalian oocytes requires the introduction of human chorionic gonadotropin (hCG) hormone in the culture media (10,11). Prior investigations have shown that the addition of human chorionic gonadotropin (hCG) hormone to the culture medium primarily enhances the function of luteinizing hormone (LH), leads to an increase in glutamine metabolism in the oocyte, and alters the distribution of calcium ions in the cytoplasm (12).

## 2. Materials and Methods

From local abattoir in wasit province ovaries from sheep were collected and transported to the laboratory in solution of normal saline (NaCl 0.9%) that contained and supplied with antibiotics (100 μg/mL streptomycin and 100IU/mL penicillin) and kept at 30-37 °C within 3 hours. The follicular liquid with the cumulus–oocytes complexes (COCs) was taking by a sterile disposable 5 mL syringe supplied by 20-gauge hypodermic needle. The immature oocytes washed three times in culture medium and transfer to maturation medium. The study groups were divided according to concentrations of gonadotropins (PMSG and hCG) (MSD Intervet, Netherland) as the following:

Control group: culture media without hCG and PMSG.

Group 1: (2 IU/mL hCG) and (2 IU/mL PMSG).

Group 2: (5 IU/mL hCG) and (5 IU/mL PMSG).

Group 3: (10 IU/mL hCG) and (10 IU/mL PMSG).

The oocytes incubated in temperature of 38C° and 95% humidity with 5% CO2 for 24 hours (13). The incubated oocytes examined under light microscope. Cumulus expended and appearance of the first polar body gives indicator for in vitro maturation of oocytes (14). The oocytes with homogenous cytoplasm, intact plasma membrane and zona pellucida were considered as normal morphology in the study. Viability test done by staining the oocytes with the trypan blue stain and classified as the following the unstained oocytes classified as live and fully stained oocytes classified as dead (15).The viability test was done after IVM immediately.

Ethical Considerations: ethical approval was not needed in this study because sheep ovaries were obtained from local abattoir.

**Statistical analysis**

The data of this study analyzed statistically by using (SPSS/PC) program version 20 software (SPSS, Chicago). The test chi square was applied to reveal the significant comparison among percentages in this study. Where letters that similar to each other consider non significant difference ($P>0.05$) between groups while letters that different to each other consider significantly difference ($P<0.05$).

## 3. Results

In the present study (100) sheep immature oocytes were tested for viability, morphology and in vitro maturation, (25) oocytes for each group. Figure (1) shown that the percentage of oocytes viability was highest in group 3 (88%), while the lowest percentage was for control group (60%) also the results appeared that the percentage of oocytes viability for group 1 (64%) and for group 2 (76%) respectively. Group 3 group appeared a significant difference ($P<0.05$) in oocyte viability while compared with control group

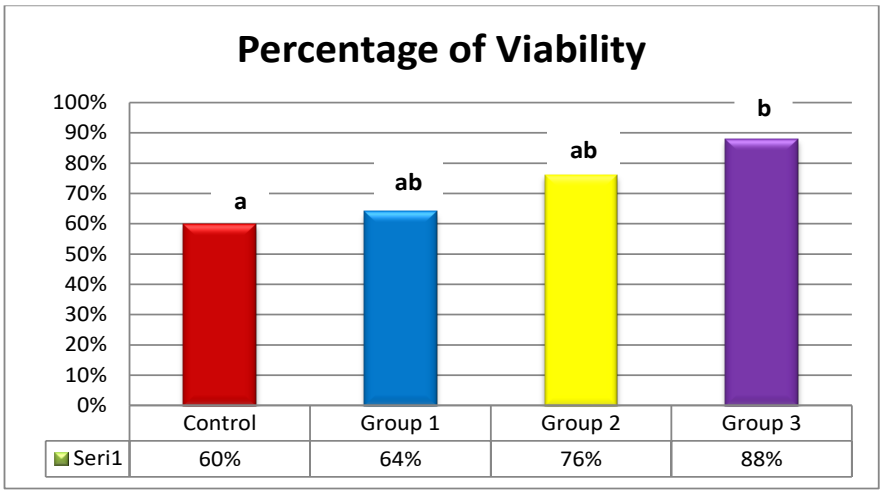


**Figure (1): Percentage of oocytes viability after *in vitro* maturation**
*** Letters that different consider significantly difference (P<0.05).**

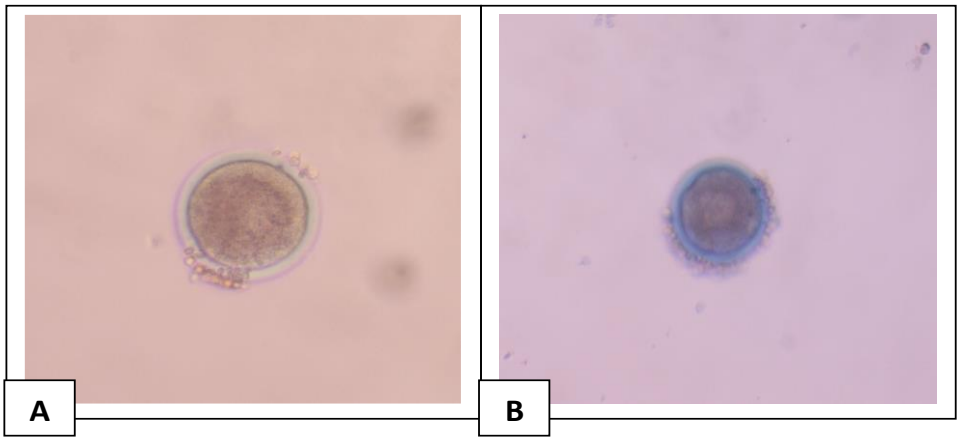


**Figure (2): A: Viable oocyte after IVM B: Non-viable oocyte after IVM**

According to this study the best percentage of normal oocytes morphology (92%) was in group 3, followed by group 2 (80%) and group 1 (68%), while the lowest percentage of normal oocytes morphology was in control group (64%). The study appeared that the normal oocytes morphology increase significantly (P<0.05) in group 3 when comparing with control group as shown in figure (3)

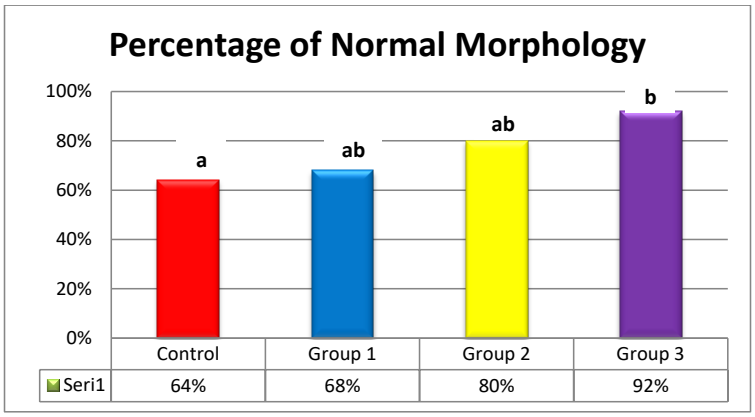


**Figure (3): Percentage of normal oocytes morphology after *in vitro* maturation.**
*** Letters that different consider significantly difference (P<0.05).**

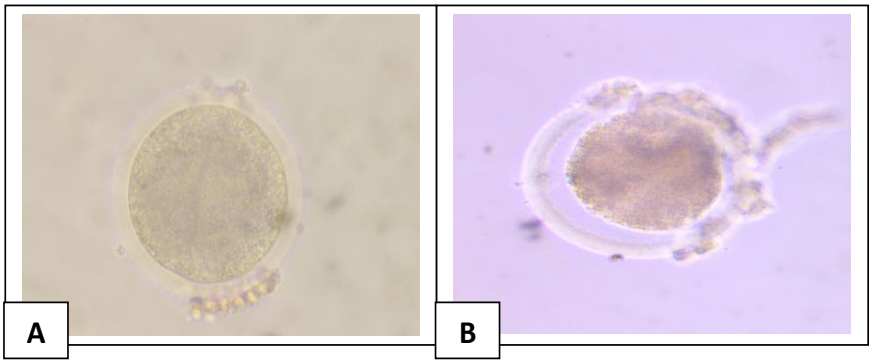


**Figure (4): A: Oocyte with normal morphology after IVM**
**B: Oocyte with abnormal morphology after IVM**

The results of in vitro maturation showed that control group appeared the lowest percentage of in vitro maturation (36%), followed by group 1 was (52%) then group 2 (64%), while group 3 appeared the highest percentage of in vitro maturation of oocytes with (76%). There was a significant decrease ($P<0.05$) in percentage of oocytes maturation appeared in control group when comparing with percentage of oocytes maturation in group 2 and group 3 as shown in figure (5).

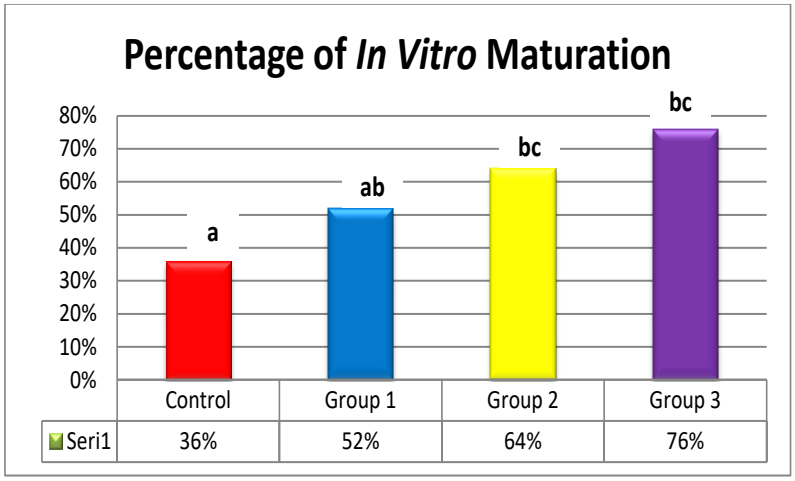


**Figure (5): Percentage of mature oocytes after *in vitro* maturation.**
*** Letters that different consider significantly difference ($P<0.05$).**

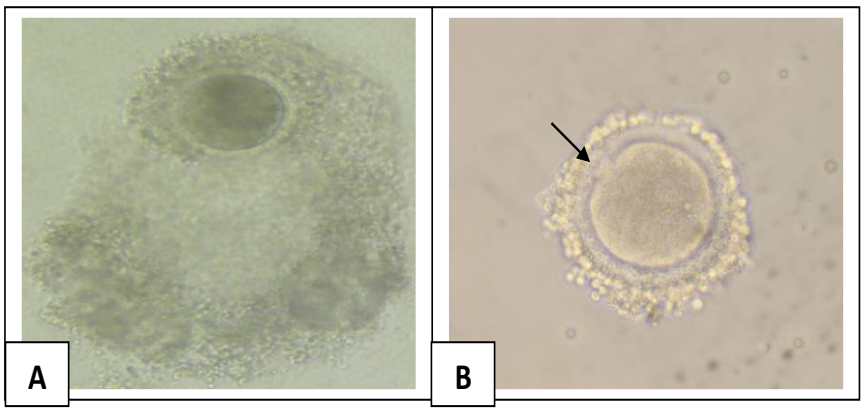


**Figure (6): A: mature Oocyte with cumulus cells expansion after IVM.**
**B: Present of polar body in mature oocyte after IVM.**

## 4. Discussion

The selection of the culture system for in vitro maturation of immature oocytes is a crucial determinant in controlling the quality and quantity of oocytes that successfully develop in vitro. The elements of the maturing medium and the ambient circumstances of the culture can influence and modify the regulation of meiosis in immature oocytes (16). Thus, it is crucial to optimise and prepare the culture medium that considers all the necessary elements for the in vitro maturation of oocytes (17).

The findings of this study indicate that the inclusion of gonadotropin hormones enhances the viability %, normal morphology, and intravitreal motility (IVM) of oocysts. The findings of this investigation were consistent with prior studies conducted on both bovine and human subjects (18,19). These findings corroborate the study conducted in 2018, which indicated that FSH plays a crucial role as the primary regulating gonadotropin in the expansion of cumulus cells (20).

Moore and Trounson said that the inclusion of gonadotropins in the maturation medium was shown to be beneficial in successful in vitro maturation of ovine oocytes (21). Experimental investigations conducted by Downs et al demonstrated that the addition of FSH to the culture media resulted in an elevation of cAMP content, thereby promoting meiotic arrest (22). The work conducted by Turathum et al. showed that the development of cumulus cells is essential for meiotic maturation since it disrupts the communicative junction, resulting in a decrease in the concentration of cAMP in the oocyte (23).

## 5. Conclusion

The findings of this study highlight the significant impact of gonadotropin hormones on the viability, morphology, and in vitro maturation of sheep oocytes. The results demonstrated that higher concentrations of gonadotropins, specifically hCG and PMSG, led to increased oocyte viability and normal morphology, with the group receiving the highest concentration showing the most pronounced effects. These findings underscore the critical role of optimized hormone concentrations in improving in vitro maturation outcomes, suggesting that the careful adjustment of gonadotropin levels could enhance the efficiency of reproductive technologies in livestock. The implications of this study are particularly relevant for advancing in vitro fertilization techniques, potentially leading to better reproductive success rates in mammalian species. Future research should explore the long-term developmental competence of oocytes matured under these optimized conditions and investigate the underlying molecular mechanisms that drive these improvements.

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