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EFFECT OF DOPAMINE ON IN VITRO MATURATION, FERTILIZATION AND DEVELOPMENTAL COMPETENCE OF BOVINE OOCYTES

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ABSTRACT

Proteins and other nitrogenous substances, such as polyamines, catecholamines, and nitric oxide require amino acids for their synthesis. The purpose of this study was to investigate the effect of dopamine supplementation in IVM (*in vitro* maturation) medium on fertilization and developmental rates of bovine oocytes. Ovaries with apparently normal reproductive organs were collected from cattle within 30 minutes of slaughter. Cumulus oocyte complexes (COCs) were collected by aspirating medium sized ovarian follicles (4-8 mm). Good quality oocytes were selected, washed and incubated in TCM199 supplemented with dopamine. Different concentrations of dopamine (0, 5, 10, 20 and 40 ng) were added to the IVM media. Oocytes were incubated for 20:22 hours at 38.5 C◦ under 5% CO2 in air with 90% humidity. A non-significant increase (P>0.05) in maturation, fertilization and developmental rates of bovine oocytes was observed when the maturation medium was supplemented with all concentrations used (5, 10, 20, and 40) ng of dopamine. Dopamine non-significantly improves IVM, IVF (invitro fertilization) and developmental rates.

***Key words**:* Bovine, Dopamine, Maturation, Fertilization

INTRODUCTION

Advancements in reproductive biotechnologies have significantly enhanced the productive efficiency and quality of livestock products (Sharawy *et al.,* 2023). Among these technologies, in vitro fertilization (IVF), has emerged as a valuable tool for selecting and breeding genetically superior animals (Sirard, 2018). A critical step in IVF is the in vitro maturation (IVM) of oocytes, which directly influences fertilization success and subsequent embryonic development. Various physiolo-gical factors, including neurotransmitters, play a significant role in regulating oocyte maturation and early embryogenesis. Among these, dopamine, a catecholamine neurotrans-mitter, has gained attention for its potential influence on reproductive processes (Satué *et al.,* 2020).

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Dopamine plays a crucial role in reproductive processes, primarily through its interaction with dopamine receptors on the ovarian follicle and oocyte (Venegas-Meneses *et al.,* 2015). It also contributes to early embryonic development by enhancing the synthesis of amino acid transporters (Elmetwally *et al.,* 2018). Ovarian follicular neurons are predominantly catecholaminergic, with dopamine concentrations increasing as the oocyte matures (Mayerhofer *et al.,* 1998; D'Albora *et al.,* 2002). Additionally, dopamine interacts with granulosa cells, influencing the secretion of key hormones such as estradiol and progesterone, which are essential for oocyte maturation (King *et al.,* 2005). Once transferred from the follicular fluid into the oocyte, dopamine is metabolized into norepinephrine (Bodis *et al.,* 1992). Studies suggest that genes responsive to neurotransmitters like dopamine, glycine, and GABA serve as potential markers of oocyte maturation in cumulus cells (Devjak *et al.,* 2012).

A key mechanism by which dopamine regulates oocyte maturation involves its impact on cyclic AMP (cAMP) levels. By binding to D2-like receptors, dopamine reduces cAMP concentrations, facilitating the resumption of meiosis and promoting oocyte maturation (Yoshida *et al.,* 2004). However, its effects on reproduction vary across species. In amphibians and fish, dopamine primarily inhibits gonadotropin release, thereby indirectly affecting oocyte maturation (Vu and Trudeau, 2016; Levavi-Sivan, 2018; Trudeau *et al.,* 2022).

Beyond its role in oocyte maturation, dopamine influences early embryonic development by modulating oxidative stress and apoptosis (Juárez Olguín *et al.,* 2016). High oxidative stress levels can impair embryonic viability, while dopamine's antioxidant properties help mitigate such damage, promoting normal development (Ramos-Ibeas *et al.,* 2020). Dopamine also regulates intracellular signaling pathways, such as MAPK and PI3K/AKT, which are critical for cell proliferation and survival during early embryogenesis (Burke, 2010).

Several studies highlight the importance of dopamine in embryo–fetal development, particularly in motor and cognitive neurological programming. Changes in the oocyte's maturation environment can significantly impact its competence, as reflected in morula and blastocyst yields following IVF (Bavister *et al.,* 1992).

So, this study investigated whether dopamine supplementation in IVM media influences bovine oocyte maturation, fertilization, and developmental competence.

MATERIAL AND METHODS

This study was conducted in Animal Reproduction Research Institute, Giza, Egypt during the period from December 2022 to December 2024. The chemicals used in this study were purchased from Sigma Chemical Co (St. Louis, MO, USA).

1. Recovery of immature oocytes

Ovaries from cattle with apparently normal reproductive organs of unknown age and breeding history were collected within 30 minutes after slaughter at the private EL-Bagor abattoir. The ovaries were kept in a thermos flask containing warm normal saline (0.9% NaCl) supplemented with gentamycin and transported to the laboratory within 1-2 hours (El-Naby *et al.,* 2013).

Upon arrival at the laboratory, the ovaries were washed in warm normal saline to remove blood and debris and then kept in a water bath at 37°C during oocyte collection (Raghu *et al.,* 2002).

Medium-sized follicles (3-8 mm in diameter) were aspirated using an 18 – gauge needle attached to a 10 ml syringe, and the contents were pooled in a 15 ml

conical tube (Neglia *et al.,* 2003). The tubes were left undisturbed for 10-15 minutes to allow the follicular cells to settle. Approximately 5 ml of the sediment was recovered using a dropper pipette and placed in an 80 mm diameter sterile Petri dish (Raghu *et al.*, 2002)
The cumulus oocyte complex (COCs) were classified into four grades (A, B, C and D) based on their morphological appearance, as described by Elmetwally *et al.* (2018).

2. Maturation of Oocytes

Grade A and B COCs were washed 3 times in TCM199 and divided into five groups:
Group (1) oocytes matured in TCM-199,
Group (2) oocytes matured in TCM-199 to which 5 ng dopamine was added,
Group (3) oocytes matured in TCM-199 to which 10 ng dopamine was added,
Group (4) oocytes matured in TCM-199 to which 20 ng dopamine was added and
Group (5) oocytes matured in TCM-199 to which 40 ng dopamine was added.

Culture dishes were covered with sterile mineral oil and incubated for 22-24 hours at 38.5 C◦ under 5% of CO2 and 90% humidity (Mehmood *et al.,* 2011).

Maturation was assessed based on cumulus expansion and nuclear maturation. Cumulus expansion was evaluated under stereo microscope. Nuclear maturation was assessed by fixation and staining using aceto-orcein stain (1% (w/v) orcein in 45% acetic acid). The stained oocytes were examined under phase contrast microscope for germinal vesicle breakdown (Nandi *et al.,* 1998).

3. Semen preparation and Fertilization

Bovine frozen semen was obtained from Animal Reproduction Research Institute and prepared for capacitation, according to Neglia *et al.* (2003).

Mature oocytes were washed 3 times in sperm-Tyrode's Albumin Lactate Pyruvat **(**TALP), then added to the fertilization drops (10 oocytes per drop). Gametes were co-incubated at 38.5 C◦ under 5% of CO_2 and 90% humidity for 4 hours. Presumably fertilized oocytes were further cultured in TCM 199 for 24 hours under the same conditions, and examined for cleavage. Fertilization was assessed by the cleavage of fertilized oocytes to 2- or 4- cell stage (Gasparrini *et al.,* 2008).

4. Developmental Stages

Presumptive zygotes (10 zygote/50 µl droplets) were cultured at 38.5 C° under 5% of CO_2 and 90% humidity, with replacement of the culture medium by fresh medium every 48 hours (Badr, 2009; Chauhan *et al.,* 1998).
Embryos were checked every 48 hours for up to 7 days to assess development to the morula or blastocyst stages.

5. Statistical Analysis

Each experiment was replicated at least three times. Data were statistically analyzed using the ANOVA test with SAS's UNIVARIATE procedure. All results are presented as mean ± SEM.

RESULTS

The data present in Table (1) demonstrated a non-significant increase (P>0.05) in the cumulus expansion rate when the in vitro maturation (IVM) medium was supplemented with 20 ng dopamine.

Table 1: Effect of in vitro maturation medium supplementation with dopamine on cumulus expansion rate.

Treatment	No of oocytes	Expansion rate (%)
control	200	80.50±2.81
5ng	100	81±3.94
10ng	100	82±3.86
20ng	201	82.59±2.68
40ng	204	81.86±2.70

No significant difference between values (P>0.05).

Data in Table (2) indicate that supplementing the IVM medium with 20 ng of dopamine resulted in a non-significant increase in the nuclear maturation rate (P>0.05).

Table 2: Effect of in vitro maturation medium supplementation with dopamine on the nuclear maturation rate.

Treatment	No of oocytes	Maturation rate (%)
Control	100	70±4.61
5ng	100	69±4.65
10ng	100	71±4.56
20ng	100	73±4.46
40ng	101	73.27±4.43

No significant difference between values (P>0.05)

In Table (3), supplementation of the IVM medium with 20 ng dopamine led to a non-significant increase in the fertilization rate (P>0.05).

Table 3: Effect of in vitro maturation medium supplementation with dopamine on fertilization rate.

Treatment	No of oocytes	Cleavage rate (%)
control	100	40±4.92
5ng	100	39±4.90
10ng	100	40±4.92
20ng	100	43±4.98
40ng	102	42.16±4.91

No significant difference between values (P>0.05).

Table (4) revealed a non-significant increase (P>0.05) in the developmental rate to blastocyst stage when the IVM medium was supplemented with 20 ng dopamine.

Table 4: Effect of in vitro maturation medium supplementation with dopamine on blastocyst developmental rate.

Treatment	No of oocytes	Blastocyst Development rate (%)
control	100	10±3.12
5ng	100	10±3.02
10ng	100	11±3.14
20ng	100	11±3.15
40ng	102	10.78±3.09

No significant difference between values (P>0.05).

DISCUSSION

Catecholamines are small, polar, chemical compounds produced in neural ectoderm-derived cells from phylogenetically evolved mammals (Nagatsu, 2006). In the animal kingdom, these compounds serve as related hormones and neurotransmitter substances (Bloom, 1988).

Dopamine regulates several physiological and behavioral processes, including reproduction, which is mediated by the hypothalamic-pituitary-gonadal (HPG) axis in vertebrates. The release of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates pituitary gonado-tropic cells to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream. Previous studies indicated that not only dopamine, but also other catecholamines, have a significant role during early fetal development (Elmetwally *et al.,* 2018).

In the current study, the addition of 20 and/or 40 ng dopamine to the maturation media resulted in a non-significant increase in the maturation rate. This may be attributed to the limited number of oocytes used in the experiments. Despite extensive research, many parts of the developmental process remain unclear, particularly the interaction of neurotransmitters and neuropeptides with oocyte maturation and early development (Herlenius and Lagercrantz, 2001). Due to resource limitations, this study relied on previous research that provides more detailed biological and molecular insights. The main function of dopamine, as well as all neurotransmitters, depends on Ca2+ signaling as a critical modulator of oocyte physiology in mammals (He *et al.,* 2021). Basically, neurotransmitters and neuro-peptides including dopamine are connected to Ca2+ during oocyte maturation (Bootman *et al.,* 2002). Although Ca2+ signaling and hormone regulation are well studied in most phases of early

development, the relationship between neuronal signaling and Ca2+ during key reproductive events remains less understood (Bootman *et al.,* 2002).

Also, dopamine has been identified as a critical regulator of early organism development (Sanz-Ezquerro *et al.,* 2017). Chemical or hormonal messengers typically transmit these signals. Several studies have demonstrated the role of neurotransmitters and neuropeptides in processes such as oocyte maturation and embryo development (Alhajeri *et al.,* 2022).

This study highlights for the first time the effects of dopamine on the expansion rate of immature bovine oocytes. All concentrations of the dopamine resulted in the expansion of more than 80 % of immature oocytes. Neither 20 nor 40 ng of the dopamine have a detrimental effect on the oocyte expansion. It appears that normal ovulation and subsequent fertilization depend on the cumulus mass expanding to its optimal size (Chen *et al.,* 1993) . A correlation was found between the rate of development to the 2-cell stage and the extent of cumulus expansion. Hyaluronic acid (HA) is the main constituent of the expanded cumulus (Lane *et al.,* 2014), and the degree of expansion directly related to the amount of HA synthesis (He *et al.,* 2021). It has been shown that FSH stimulates the de novo production of HA *in vitro* (Vanderhyden, 1993).

Oocyte maturation is primarily stimulated by the complex of cyclin B and cyclin-dependent kinase CDK1, known as maturation promoting factor (MPF). Increased cAMP levels inhibit MPF activation in arrested oocytes. Germinal vesicle breakdown (GVBD) and chromosomal segregation are initiated when cAMP levels decrease, accompanied by an increase in MPF activity (Adhikari and Liu, 2014).

The present results regarding the effect of dopamine on the expansion of immature bovine oocytes are supported by substantial evidence that neurotransmitters, neuro-peptides and calcium play a crucial role in oocyte maturation and early development. Neurotransmitter receptors are categorized into two types: ionotropic and metabotropic.

Dopamine is involved in various peripheral physiological processes, including immune system regulation, respiratory function, cardiovascular function, olfactory perception, retinal processes, hormonal regulation, and renal function regulation (Iversen and Iversen, 2007).

In the current study, we investigated the effects of the dopamine supplementation in maturation media on the cleavage and blastocyst rates of the bovine oocyte. Recent advances in assisted reproductive technologies have enabled researchers to achieve significant milestones in improving the in vitro competence of oocyte and sperm either in bovine (Elmetwally *et al.,* 2022) or in murine. Gamete competence is defined as the ability to fertilize successfully and produce a normal blastocyst capable of implanting in the uterus and resulting in healthy offspring (Elmetwally *et al.,* 2022).

Both developmental parameters showed a non-significant increase, with a higher concentration of dopamine. Catecholamine traditionally functions as signaling molecules, transmitting impulses from neuron to an effector organ or from neuron to another. However, neurotransmission may represent one of their molecules' signaling functions. As morphogens in developing embryos, they may have a more fundamental role (Lauder, 1988).

Morphogens are chemicals that influence embryonic patterning and gene activity (Briscoe and Small, 2015). Evidence for catecholamines' involvement in this process has been compiled in a number of reviews and is based on descriptive examples from a wide range of animal species at different developmental stages (Bootman, 2002). For instance, dopamine appears to regulate the

development of synaptic target neurons in the rodent corpus striatum. These previous studies suggest that dopamine plays an important role during the early development of the fetus within the uterus.

CONCLUSION

In conclusion, dopamine non-significantly improves IVM, IVF and developmental rates.

Ethical approval
This study was conducted according to the standards of the Research Ethics Committee M /90 Faculty of Veterinary Medicine, Mansoura University, Egypt.

Availability of data and materials
The data that support the results of this study is available within the article.

Competing interests
The authors declare no competing interests.

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تأثير الدوبامين فى نضوج وإخصاب وقدرة بويضات الأبقار علي النمو

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تتطلب البروتينات والمواد النيتروجينية الأخرى مثل البوليامينات والكاتيكولامينات وأكسيد النيتريك أحماض أمينية لتخليقها. الغرض من هذه الدراسة هو دراسة تأثير إضافة الدوبامين إلى وسط النمو على نضوج بويضات الأبقار و إخصابها ومعدلات نموها. تم جمع المبايض من الأعضاء التناسلية الطبيعية للماشية خلال ٣٠ دقيقة من ذبح الحيوانات. تم جمع مجمعات البويضات الركامية عن طريق شفط بصيلات المبيض متوسطة الحجم (٤-٨ مم). تم اختيار البويضات ذات النوعية الجيدة وغسلها واحتضانها في TCM199 مع إضافة الدوبامين. تمت إضافة تركيزات مختلفة من الدوبامين (٠، ٥، ١٠، ٢٠ و ٤٠ نانوغرام) إلى وسائط النضج المعملي IVM. تم تحضين البويضات لمدة ٢٢:٢٠ ساعة عند ٣٨,٥° درجة مئوية تحت ٥٪ ثاني أكسيد الكربون في الهواء مع رطوبة ٩٥٪. كانت هناك زيادة غير معنوية في معدلات النضج والإخصاب والنمو لبويضات الأبقار عندما تم تزويد وسط النضج بـ ٢٠ نانوغرام من الدوبامين. الدوبامين يحسن بشكل غير ملحوظ النضج المعملي IVM، والاخصاب المعملي IVF ومعدلات النمو.