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Effect of histidine and L-Tyrosine supplementation in maturation medium on in-vitro developmental outcomes of buffalo oocytes

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Abstract

The present study was designed to investigate the effects of amino acid (histidine and L-Tyrosine) on in vitro maturation (IVM), in vitro fertilization (IVF), cleavage (CR) rates, and in vitro embryonic cultivation (IVC; Morula and Blastocyst stage) in buffaloes. Within two hours of buffalo slaughter, the ovaries were collected and transported to the laboratory. Follicles with a diameter of 2 to 8 mm were aspirated to recover the cumulus oocyte complexes (COCs). Histidine (0.5, 1, and 3 mg/ml) or L-Tyrosine (1, 5, and 10 mg/ml) were added to the synthetic oviductal fluid (SOF) and Ferticult media. The IVM, IVF, CR, and IVC (Morula and Blastocyst) rates were evaluated. The results showed that SOF maturation media containing histidine at 0.5 mg/ml significantly ($P \leq 0.01$) improved the oocyte maturation when compared to control and other concentrations. The addition of histidine to FertiCult media at 0.5, 1, and 3 mg/ml did not improve the IVM, IVF, CR, or IVC percentages. However, the embryos in the control group were unable to grow into a morula or blastocyst in the SOF or Ferticult, while addition of L-Tyrosine to the SOF or Ferticult at various concentrations improved IVC (morula and blastocyst rates). There was a significant ($P \leq 0.01$) increase in IVM when histidine was added to SOF medium at a concentration of 0.5 mg/ml compared with L-Tyrosine. Also, there were significant ($P \leq 0.01$) increases in IVC when L-Tyrosine was added to SOF medium at concentrations of 1 and 10 mg/ml compared with histidine. In conclusion, the supplementation of the SOF and FertiCult with the amino acids histidine and L-Tyrosine improve the maturation rate of oocytes and development of in vitro-produced buffalo embryos.

Keywords IVM, IVF, IVC, Histidine, L-Tyrosine, SOF, Ferticult, Buffalo oocytes

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Introduction

Water buffaloes (*Bubalus bubalis*) are important in rural livestock production. The buffalo population in Egypt was predicted to be 3.7 million, producing around 45% (4.5 million tonnes/year) and 19% (367 thousand tonnes/year) of total milk and meat output, respectively [1]. On the other hand, buffaloes are known to have a lower reproductive performance than cattle due to late puberty, weak estrus expression, and extended inter-calving intervals therefore, an increase in culling rate, costs of fertility treatment, and number of inseminations, consequently increasing the cost of each unit of milk that is produced [2].

In vitro embryo production (IVEP) has been introduced and applied to improve the poor reproductive efficacy of buffaloes [3]. Also, IVM is a critical component of IVEP, facilitating the utilization of a large number of oocytes inside an ovary [4]. However, only a small proportion of in vitro matured oocytes show full developmental potential to term [5]. Furthermore, it is likely that buffalo oocytes and embryos are very sensitive to oxidative stress due to their high lipid content [6]. One of the first media designed for this purpose was synthetic oviduct fluid (SOF), which was established from the composition of bovine oviductal fluid SOF medium [7], which was originally based upon the biochemical and physiological analysis of sheep oviductal fluid [8], has been used prasadin the IVM of oocytes in bovine [9], goat [10] and in other species. Furthermore, buffalo oviducts secretions in particular the oviductal specific glycoproteins varied with cyclic stages and may affect the fertilization and early embryonic development [11, 12]. In human IVF, FertiCult is a chemically balanced salt solution (HEPES buffered) that also contains gentamycin, sperm washing, embryo replacement, and the sperm swim-up procedure. It was reported that Ferticult medium enhanced cumulus cell expansion and nuclear maturation more than tissue culture medium-199 (TCM-199), Ham's F-10 medium, or minimum essential medium (MEM) in buffalo oocytes [12].

Amino acids can be categorized as either essential or non-essential. Essential amino acids are unable to be synthesized de novo and must be obtained from the diet, such as histidine and lysine [13], whereas nonessential amino acids are not required in the diet as they can be synthesized in vivo, such as alanine. However, some nonessential amino acids have been found to be essential in certain situations and disease states. These amino acids are called conditionally essential and must be obtained from the diet, such as tyrosine [14]. Thus, amino acids are involved in several physiological processes that ensure the maintenance of cellular homeostasis. The major concern with the inclusion of amino acids in embryo culture medium is the production of toxic ammonium. Thus, to prevent the spontaneous breakdown of amino acids, medium should be stored at 4 °C. Moreover, care should be taken to limit the amount of time the medium is exposed to 37 °C prior to the addition of the embryo. If media are handled appropriately, amino acids impart immense metabolic and homeostatic benefits to the developing embryo [15]. The supplementation of amino acids to SOF medium may increase the pool size of endogenous amino acids and denovo protein synthesis [16]. Also, adding L-Tyrosine to FertiCult could improve the IVM and IVF in buffaloes.

To the best of our knowledge, there are scarce reports on the effect of essential amino acid addition on oocyte maturation as well as embryo cultivation media in buffaloes. The aim of the work was to evaluate the effect of amino acid addition (Histidine and L-Tyrosine) to the oocyte maturation medium on IVM, IVF rates, and embryonic development in buffaloes.

Materials and methods

The present study was carried out in the Department of Theriogenology, Faculty of Veterinary Medicine, Aswan University, Egypt.

Collection of buffalo ovaries

Ovaries from apparently normal reproductive organs of adult buffaloes slaughtered in a local abattoir in Aswan were collected within 30 min of slaughtering. The specimens were transported within two hours from slaughtering to the laboratory in a thermal flask containing normal physiological saline (0.9% NaCl) fortified with penicillin (400 IU/ml) and streptomycin (50 µg/ml) and maintained at 37 °C [17].

Media used in the experiment

The aspiration medium used was medium-199 with Earle's salts, L-glutamine, and 25 mM HEPES, supplemented with 3% (v/v) heat-inactivated calf serum (heat-treated at 56 °C for 30 min) and antibiotics (1 µg/ml Gentamycin). It was kept in the refrigerator at 5 °C until used. Two maturation media were used, the first was synthetic oviductal fluid (SOF), freshly prepared in the laboratory and stored in a refrigerator at 5 °C until used. SOF was supplemented with 10% fetal calf serum (FCS) along with gentamycin. The second one, FertiCult medium (FertiPro N.V., Belgium), was obtained in liquid form and stored in a refrigerator at 5 °C until used. FretiCult is characterized by pH: 7.3–7.6; Osmolarity: 270–290 mOsm/kg; Sterility: sterile; Endotoxin: <0.25EU/ml; Mouse embryo test: > 80% blastocysts after 96 h incubation; Shelf life: 18 months from date of production. All media used in the present work were filtered using a 0.2 µm (Millipore, USA) syringe filter and incubated for at least 2 h in

a humidified atmosphere (95%) under 5% CO_2 at 38 °C before culturing the oocytes.

Cumulus oocyte complexes collection

In the laboratory, the ovaries were washed several times in a warm 0.9% NaCl solution at 37 °C until obtaining clear saline free from blood and then kept in water bath at 37 °C during oocyte collection. The ovaries were dried lightly with sterile paper towels, COCs were recovered from visible ovarian surface follicles (2–8 mm) with the help of an 18-gauge needle attached to a 10 ml syringe in aspiration medium. Only COCs with more than five compact layers of cumulus cells, and homogeneous cytoplasm and intact zona pellucida were used for in vitro maturation (IVM) in vitro fertilization (IVF) [18].

In vitro maturation of buffalo oocytes with supplementation of histidine and L-Tyrosine

Once COCs located in the Petri dish, they were recovered with a sterile glass pipette of suitable diameter and transferred into another dish containing fresh prewarmed washing medium. The glass pipette used in picking oocytes had a pore diameter wide enough to avoid disruption of the cumulus cells surrounding the oocytes. Selected oocytes were washed four times in fresh prewarmed washing medium followed by one final washing in IVM medium before being transferred to in vitro maturation medium droplet. The oocytes (10–20) were placed in 50–100µL droplet of maturation medium in a four- well culture plate (Falcon, USA), covered with sterilized light mineral oil (Sigma, USA). The culture dishes were placed in a CO_2 incubator (95% relative humidity, 5% CO_2 at 38°C) for 24 h.

The oocytes were divided into four groups during maturation as follows: Group 1: Histidine was added to the SOF medium with different concentrations (control, 0.5, 1, 3 mg/ml); Group 2: Histidine was added to the Ferticult medium as in group1; Group 3: L-Tyrosine was added to the SOF medium with different concentrations (control, 1, 5, 10 mg/ml); Group 4: L-Tyrosine was added to the Ferticult medium as in group3 (Fig. 1).

After 24 h of oocyte incubation, the maturation rate was assessed according to the degree of cumulus cell expansion [19] under stereomicroscope (Fig. 2A). Oocytes were examined for polar body extrusion (PBE) by identifying them in the perivitelline space after staining the oocytes. Some oocytes were freed from cumulus cells using 0.1% hyaluronidase, then fixed in a 1:3 aceto-ethanol solution and dehydrated with absolute ethanol, then stained with 1% aceto-orcein (Merck & Co., Inc., Whitehouse Station, NJ, USA), and de-stained with aceto-glycerol (glycerol: acetic acid: distilled water = 1:1:3 v/v) as described [20]. Oocyte nuclear maturation was evaluated with a compound microscope at 200 to 400X. Both cumulus expansion and PBE were evaluated to assess the maturation rate [3, 21].

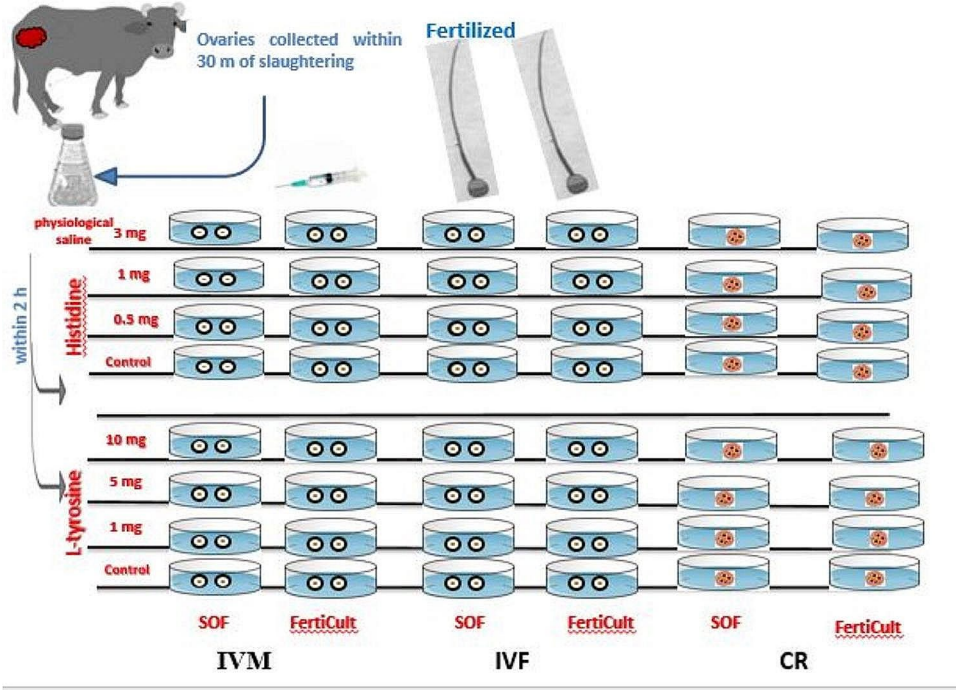

Fig. 1 Illustrative diagram showing the experimental design, IVM, in vitro maturation;, IVF, in vitro fertilization; CR, cleveage rate (morula, blastocyst)

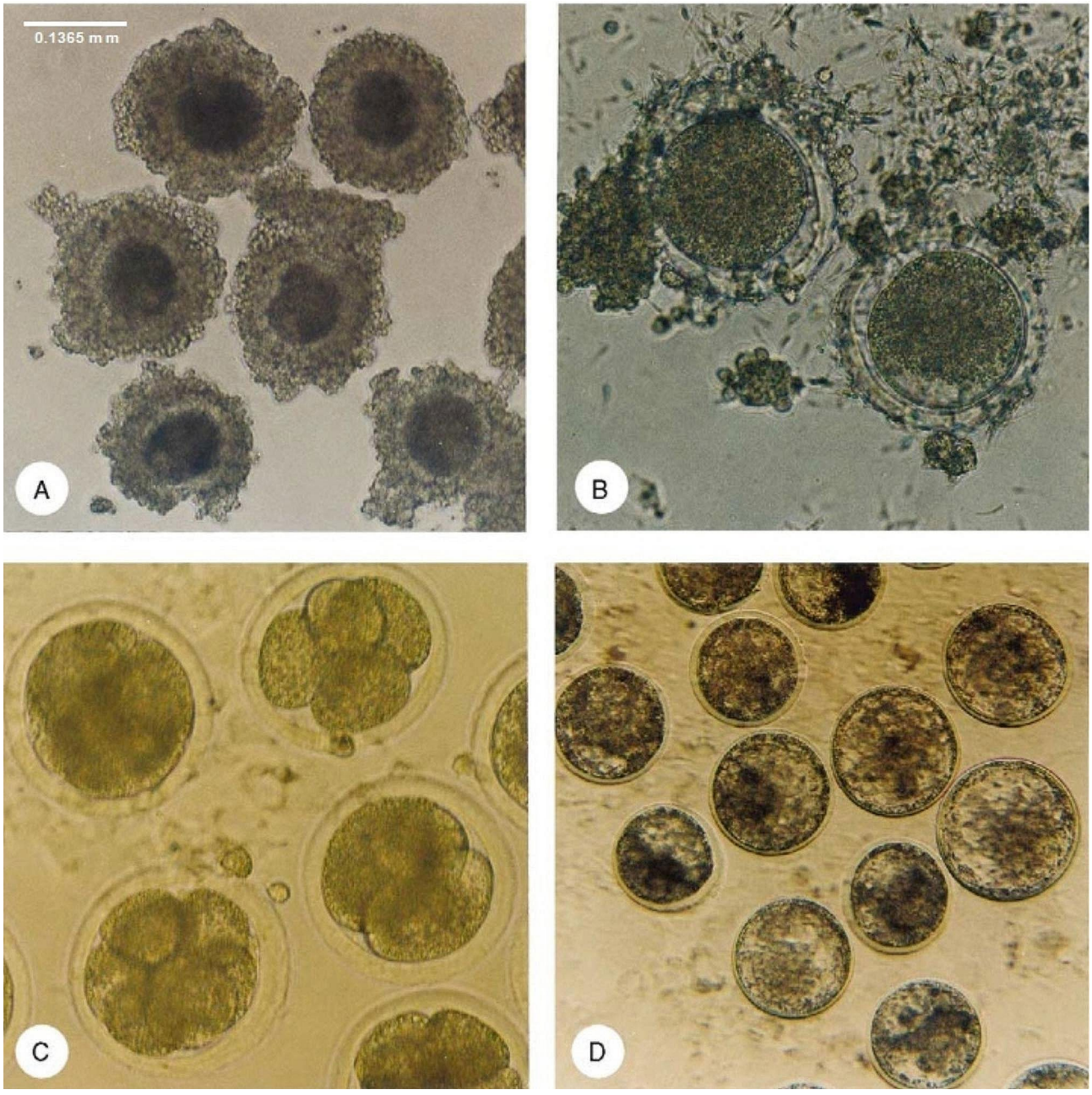

Fig. 2 (**A**) Mature oocytes with cumulus expantion; (**B**) Penetration of sperm to oocyte during in vitro fertilization, (**C**) Cleavage of embryos 4 – 1 cell stages, (**D**) Morula and early blastocyst

In vitro fertilization procedures

Frozen semen straws from fertile buffalo bulls previously examined and kept in liquid nitrogen (-196 °C) obtained from the Artificial Aswan Veterinary Directorate were used in this study. Sperm TALP (SP-TALP) was used for washing spermatozoa for selection with the swim-up technique. The selected spermatozoa were washed twice by centrifugation (x500 g/10 min). The sperm pellet was reconstituted with the fertilization TALP (F-TALP) medium. The prepared spermatozoa were capacitated by incubation in the CO_2 incubator in 38.5°, 5% CO_2 for two hours before further use [21]. Matured oocytes were partially denuded from the surrounding cumulus cells to allow easy penetration of the sperm cells. They were washed twice in pre-warmed IVF medium (F-TALP). A total of 15–20 matured oocytes were placed in each well of the culture dish containing 50µL of fertilization medium to which 20µL of sperm suspension was added (2×10^6 sperm cells/ml) (Fig. 2B). A layer of 200µL of sterile liquid light mineral oil was placed to cover the

sperm-oocytes mixture then incubated for 18–20 h at 38.5°C under 5% CO_2 in humidified air [22]. After 18–20 h of the oocytes sperm co-incubation, the presence of first and second polar bodies was detected under the microscope. The fertilization rates were recorded as:-

$$Fertilization\ Rate\ (\%) = \frac{No.\ of\ Fertilized\ Oocytes}{Total\ No.\ of\ matured\ cultured\ Oocytes} X\ 100$$

At the end of gametes co-incubation, few oocytes were freed of the attached cumulus cells, fixed in acetic acid-ethanol (1:3), stained with 1% aceto-orcein stain and examined under phase-contrast microscope (×400) for assessing the in vitro fertilization rate [23].

In vitro culture of embryos

After fertilization, adhered cumulus cells to the zona pellucida were removed mechanically by repeated pipetting and washed four times in modified SOF medium (mSOF) supplemented with 10% FCS+50 µg/Genatmycin which was prepared in the laboratory [3, 8]. The presumptive zygotes were transferred in in vitro culture droplets (100 µl of IVC media) for further development in CO_2 incubator under 5% CO_2 at 38 °C and humidified air. Zygotes were examined for cleavage after forty-eight hours of treatment. At 72 h post-treatment, all cleaved embryos were transferred to replacement medium (same as IVC medium except that BSA was replaced with 10% FBS) at 72 h post-treatment and maintained for 8 d at 5% CO_2 and 38.5 °C with the replacement of medium after every 48 h. Oocytes that had not cleaved (unfertilized) were removed from the culture droplets, leaving only those that had undergone cleavage. During the observation period, gentle shaking of the culture dish was done to allow a uniform environment among fertilized ova. The culture medium was replaced with a fresh one (pre-incubated for at least 2 h inside the incubator) prior to culture of fertilized oocytes for 7 days [24]. Developmental competence was assessed by evaluating cleavage, morula and blastocyst rates on days 2, 5 and 7, respectively [25], (Fig. 2C & D).

Statistical analysis

Analysis of data was done with SPSS Inc.'s Statistical Package for Social Sciences version 20 (Chicago, IL, USA). Rates of oocyte maturation, IVM, IVF, CR, and embryo production IVC (morula, and blastocyst) were calculated using the Chi-square for trend. $P<0.05$ was used to determine the level of significant difference.

Results

The effects of histidine supplementation in various concentrations to in vitro maturation media (SOF and FeriCult) on oocyte maturation rate, following fertilization rate, and developmental competence rates (cleavage, morula, and blastocyst) are presented in Table 1.

Replenishing the SOF maturation media with histidine at a concentration of 0.5 mg/ml significantly ($P\leq0.01$) enhanced the IVM of oocytes (108/143; 75.5%) compared to the control (15/32; 46.9%) and other groups supplemented with a higher concentration of histadine. At the same concentration of histadine (0.5 mg/ml) addition to SOF fertilization media the CR and the embryonic development (morula and blastocyst) were found to be higher than any other experimental group (Table 1). Although, the addition of histadine to FertiCult media at 0.5, 1 and 3 mg/ml did not enhance the percentages of IVM, IVF, CR, and embryo development (morula, blastocyst) in the treated groups compared to each corresponding control (Table 1), however, all treatment groups showed embryonic development upto blastocyst stage.

The data related to the effects of L-Tyrosine replenishing at different concentrations in SOF and FeriCult media on oocytes maturation, fertilization rate and subsequent developmental competence (cleavage, morula, and blastocyst) are summarized in Table 2. There were no significant differences in IVM, IVF, and CR rates between the treatment and control groups after adding L-Tyrosine to both SOF and FertiCult. The embryos in the control groups were unable to develop into a morula or blastocyst in the SOF and Ferticult, whereas adding L-Tyrosine to both maturation medium in various concentrations improved embryonic development upto morula and blastocyst stage (Table 2).

The data concerning the effects of different concentrations of Histidine and L-Tyrosine to SOF and FertiCult media on IVM, IVF, CR and IVC regardless to control groups are demonstrated in Tables 3 and 4. There was a significant ($p\leq0.01$) increase in IVM when histidine was added to SOF medium at concentration 0.5 mg/ml (75.5%) compared with L-Tyrosine. Also, the result showed significant ($p\leq0.01$) increases in IVC when L-Tyrosine was added to SOF medium at concentration 1 and 10 mg/ml (12/18, 66.7% and 8/12, 66.7, respectively) compared with histidine.

There was a significant ($p\leq0.05$) increase in IVM rate when histidine was added to FertiCult medium at concentration 0.5 mg/ml (63/102, 61.8%) compared with L-tyrosine. Also, there were significant ($p\leq0.05$) increases in IVC rates when L-Tyrosine was added to FertiCult medium at concentrations 1.0 and 10 mg/ml (12/32, 75.0% & 13/31, 68.4%, respectively) compared with L-Tyrosine. There were non-significant ($P\geq0.05$) differences between different concentrations of histidine

Table 1 Effects of histidine amino acid (0.5, 1, 3 mg/mL) addition to (SOF) and FeriCult culture media on the IVM, IVF, CR and embryo stages morula and blastocyst development.in buffaloes

Items		SOF				
		Control	0.5 mg/mL	1 mg/mL	3 mg/mL	Chi (*P*-value)
IVM	*N*	15/32	108/143	46/77	51/79	12.467 (0.006)**
	%	46.9	75.5	59.7	64.6	
IVF	*N*	5/15	53/108	19/46	24/51	1.811 (0.613)^{NS}
	%	33.3	49.1	41.3	47.1	
			101			
CR	*N*	3/5	28/53	10/19	10/24	1.079 (0.782)^{NS}
	%	60	52.8	52.6	41.7	
Morula	*N*	1/5	9/28	2/10	2/10	4.045 (0.671)^{NS}
	%	33.3	32.1	20	20	
Blastocyst	*N*	0	3/9	0	0	
	%	0	10.7	0	0	
		FeriCult				
		Control	0.5 mg/mL	1 mg/mL	3 mg/mL	Chi (*P*-value)
IVM	*N*	11/21	63/102	53/89	51/85	0.647 (0.886)^{NS}
	%	52.4	61.8	59.6	60	
IVF	*N*	7/11	26/63	32/53	26/51	4.954 (0.175)^{NS}
	%	63.6	41.3	60.4	51	
CR	*N*	3/7	12/26	19/32	12//26	1.565 (0.667)^{NS}
	%	42.9	46.2	59.4	46.2	
Morula	*N*	1/3	5/12	9/19	6/19	4.585 (0.598)^{NS}
	%	33.3	41.7	47.4	31.5	
Blastocyst	*N*	0/3	1/12	5/19	3/12	
	%	0	8.3	26.3	25	

SOF = Synthetic oviductal fluid. FertiCult™ IVF medium is intended for washing and holding of human oocytes. The differences were considered significant at ***$P<0.001$; ^{NS} stands for non-significant difference. The values denotes sum of three experiments. Chi-square (Chi^2) for the trend of the morula and blastocyst production rates was calculated considering the sum of values scored for the three experiments

and L-Tyrosine on in IVF and CR rates and cultivation rates when added to FertiCult media (Table 4).

Discussion

In the current study, the IVM and IVF of buffalo oocytes and cleavage and embryo development rates after supplementation of SOF and FertiCult media with amino acids viz. histidine and L-Tyrosine were found enhanced. The findings agreed with the previous studies that recorded the beneficial effects of amino acid addition to the culture medium in comparison to the control [26]. The present study confirmed that the buffalo oocyte's maturation needs amino acids for embryonic development from zygote to blastocyst. Moreover, it was noticed that supplementation of the culture medium with amino acids decreased the percentage of embryos arrest during culture which might be due to the increased endogenous amino acid pool and/or de novo protein synthesis [27]. Furthermore, L-Tyrosine is a precursor of two well-known biogenic amines, namely, dopamine and epinephrine that exert similar beneficial effects on in vitro maturation and in IVF [28].

Replenishing the SOF maturation medium with histidine at a concentration of 0.5 mg/ml significantly enhanced the IVM of oocytes compared to the control and other treated groups. Our results matched with those previously documented, which revealed that supplementation of amino acids improved maturation and subsequent development of bovine oocytes [29]. Also, histidine is essential in culture media because enzymes neutralize it and oocytes cannot manufacture it again [30]. On the other hand, supplementing amino acids with carbohydrates increased the maturation rate of bovine oocytes, but amino acids alone had no positive effects [31].

Although there were non-significant differences between the control and treated groups after the addition of histidine to FertiCult fertilization medium, however, there were marked embryonic developments up to the blastocyst stage in all treated groups. Another study has suggested that supplementation of amino acids, especially EAA, in chemically defined maturation media promotes bovine oocyte cytoplasmic maturation and subsequent embryonic development [32].

The embryos in the control groups were unable to develop into a morula or blastocyst in the SOF and FertiCult, while supplementation of L-Tyrosine to SOF or Ferticult at different concentrations enhanced the embryonic (morula and blastocysts) development rates. In the

Table 2 Effects of L-tyrosine amino acid (1, 5, 10 m/mL) addition to (SOF) and FeriCult culture media on the IVM, IVF, CR and embryo stages morula and blastocyst development.in buffaloes

Items				SOF		
		Control	1 mg/mL	5 mg/mL	10 mg/mL	Chi (*P*-value)
IVM	*N*	7/10	85/140	97/145	72/135	5.767 (0.123)^{NS}
	%	70	60.7	66.9	53.3	
IVF	*N*	3/7	39/85	54/97	31//72	3.17 (0.366)^{NS}
	%	42.9	45.9	55.7	43	
CR	*N*	1/3	18//39	25/54	12/31	0.679 (0.878)^{NS}
	%	33.3	46.2	46.3	38.7	
Morula	*N*	0/1	12/18	12/25	8/12	5.577 (0.233)^{NS}
	%	---	66.7	44.4	66.7	
Blastocyst	*N*	0//1	5/18	7/27	3/12	
	%	---	27.8	29.9	25	
				FeriCult		
		Control	1 mg/mL	5 mg/mL	10 mg/mL	Chi (*P*-value)
IVM	*N*	5/8	66/112	82/161	56/129	6.821445 (0.102)^{NS}
	%	62.5	58.9	50.9	43.4	
IVF	*N*	2/5	32/66	33/82	31/56	3.229 (0.358)^{NS}
	%	40	48.5	40.2	55.4	
CR	*N*	1/2	16/32	22/33	19/31	2.009 (0.571)^{NS}
	%	50	50	66.7	61.3	
Morula	*N*	0/1	12/16	14//22	13/19	4.641 (0.326)^{NS}
	%	0	75	63.6	68.4	
Blastocyst	*N*	0/1	4/16	7/22	3/19	
	%	0	25	31.7	15.8	

SOF = Synthetic oviductal fluid. FertiCult™ IVF medium is intended for washing and holding of human oocytes. The differences were considered significant at ***$P<0.001$; ^{NS} stands for non-significant difference. The values denotes sum of three experiments. Chi-square (Chi^2) for the trend of the morula and blastocyst production rates was calculated considering the sum of values scored for the three experiments

Table 3 Comparison between different concentrations of histidine and of L-tyrosine amino acid added to culture (SOF) and FeriCult media on the IVM, IVF, CR and embryo stages morula and blastocyst development.in buffaloes

Items		**Histidine**			**L-tyrosine**			**Chi (*P*-value)**
		0.5 mg	**1 mg**	**3 mg**	**1 mg**	**5 mg**	**10 mg**	
IVM	*N*	108/143	46/77	51/79	85/140	97/145	72/135	16.67 (0.005)**
	%	75.5	59.7	64.6	60.7	66.9	53.3	
IVF	*N*	53/108	19/46	24/51	39/85	54/97	31/72	4.038 (0.544)NS
	%	49.1	41.3	47.1	45.9	55.7	43.1	
CR	*N*	28/53	10/19	10/24	18/39	25/54	12/31	2.114 (0.833)NS
	%	52.8	52.6	41.7	46.2	46.3	38.7	
Morula	*N*	9/28	2/10	2/10	12//18	12/25	8/12	33.241 (0.000)***
	%	32.1	20	20	66.7	44.4	66.7	
Blastocyst	*N*	3/28	0/10	0/10	5/18	7/25	3/12	
	%	10.7	0	0	27.8	25.9	25	

SOF = Synthetic oviductal fluid. FertiCult™ IVF medium is intended for washing and holding of human oocytes. The differences were considered significant at **$P<0.01$; ***$P<0.001$; ^{NS} stands for non-significant difference. The values denotes sum of three experiments. Chi-square (Chi^2) for the trend of the morula and blastocyst production rates was calculated considering the sum of values scored for the three experiments

same line, early cleavage rate was higher in media containing high content of essential amino acids, while more cleaved ova complete its development when cultured in medium with a lower concentration of essential amino acids [33]. Margaret and John [34], found that histidine may accelerate amino acid oxidation and improve protein turnover. Also, culture for the first 72 h in nonessential amino acids and glutamine stimulated the cleavage to the morula stage and subsequent blastocyst development [35].

The addition of amino acids, which play a role in controlling osmotic pressure [36] and intracellular pH [37], to the culture medium during IVM could protect mammalian oocytes from damage in a changing microenvironment. A previous study demonstrated that oocyte maturation and male pronuclei formation competence

Table 4 Comparison between different concentrations of histidine and of L-tyrosine amino acid added to and FeriCult media on the IVM, IVF, CR and embryo stages morula and blastocyst development.in buffaloes

Items		Histidine			L-tyrosine			Chi (*P*-value)
		0.5 mg	1 mg	3 mg	1 mg	5 mg	10 mg	
IVM	*N*	63/102	53/89	51/85	66/112	82/161	56/129	12.23 (0.032)*
	%	61.8	59.6	60	58.9	50.9	43.4	
IVF	*N*	26/63	32/53	26/51	32/66	33/82	31/56	7.729 (0.172)NS
	%	41.3	60.4	51	48.5	40.2	55.4	
CR	*N*	12/26	19/32	12/26	16/32	22/33	19/31	4.514 (0.478)NS
	%	46.2	59.4	46.2	50	66.7	61.3	
Morula	*N*	5/12	9//19	6/12	12/16	14/22	13/19	17.734 (0.05)*
	%	41.7	47.4	50	75	63.6	68.4	
Blastocyst	*N*	1/12	5/19	3/12	4/16	7/22	3/19	
	%	8.3	26.3	25	25	31.8	15.8	

SOF = Synthetic oviductal fluid. FertiCult™ IVF medium is intended for washing and holding of human oocytes. The differences were considered significant at $*P<0.05$; $***P<0.001$; ^{NS} stands for non-significant difference. The values denotes sum of three experiments. Chi-square (Chi^2) for the trend of the morula and blastocyst production rates was calculated considering the sum of values scored for the three experiments

were increased by the addition of amino acids to the medium during IVM in pigs [38].

There was a significant increase in IVM when histidine was added to SOF medium at concentration 0.5 mg/ml compared with L-Tyrosine. Also, there were significant increases in IVC when L-Tyrosine was added to SOF medium at concentration of 1.0 and 10 mg/ml compared with histidine. The ATP produced via amino acid metabolism may be used as an energy source during the in vitro development of mammalian oocytes and embryos [39]. Moreover, amino acid supplementation during IVM may affect not only the maturation of immature oocytes but also their subsequent development by maintaining a favorable culture environment [40].

Conclusions

In conclusion, histidine and L-Tyrosine improve the maturation rate of oocytes as well as the development of the in vitro produced buffalo embryos. Supplementing the SOF maturation medium with histadine at a concentration of 0.5 mg/ml increased oocyte maturation substantially. Furthermore, adding histidine to FertiCult media resulted in embryonic development upto the blastocyst stage, and the addition of L-Tyrosine to SOF or Ferticult at various concentrations (1.0 and 10 mg/ml) enhanced the embryonic development upto the morula and blastocyst stages.

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Author contributions
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Data availability
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Declarations

Ethics approval and consent to participate
This study was discussed and approved by the Department of Theriogenology Faculty of Veterinary Medicine, Aswan University, Egypt. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was conducted in accordance with Institutional and National Guidelines for the care and use of animals were followed according to the OIE standards, the Ethical Committee of the Faculty of Veterinary Medicine, with the permission number (06/2023/0095).

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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