Egypt. J. Vet. Sci. **Vol. 54,** No. 6, pp. 1097-1110 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Antibiotic Resistance Microbial Contamination During In-vitro Maturation of Bovine and Camel Oocytes: Causes and Management

Shimaa T. Omara*[1], Ahmed M. Nagy[2], Mayada A. Hassan[3], Ahmed M. Farag[4], Abdel-Mohsen M. Hammam[2]

[1]*Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, 33 Bohouth St. Dokki, Affiliation I.D. 60014618, Postal Code 12311, Giza, Egypt.*

[2]*Department of Animal Reproduction and Artificial Insemination, Veterinary Research Institute, National Research Center, 33 Bohouth St. Dokki, Affiliation I.D. 60014618, Postal Code 12311, Giza, Egypt.*

[3]*Department of Artificial Insemination and Embryo Transfer Research, Animal Reproduction Research Institute (ARRI), Agricultural Research Center (ARC), Giza, Egypt.*

[4]*Department of Cattle Breeding Research, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza, Egypt.*

MICROBIAL contamination in the in-vitro embryo production (IVEP) laboratories can't be underestimated even with the strictest IVEP laboratories. The current study aimed to determine the prevalence of bacterial contamination during the in-vitro maturation (IVM) procedure in an Egyptian veterinary IVF laboratory and to associate them with specific clinical outcomes with suggestions of what could be done to minimize the impact of such an event. The study included 134 samples that were used in the IVM procedure; normal salines, cystic ovaries, large follicles, ovarian surfaces, follicular aspirates, phosphate buffer salines, tissue cultures, inoculated tissue cultures with oocytes, and paraffin oil. The samples were examined for the presence of bacterial contamination using standard morphological, microbiological, and biochemical tests. The bacterial isolates were tested for their antimicrobial resistance profiles using the Kirby Bauer disk diffusion method. A percent of 85.1 % of the examined samples were positive for bacterial contamination. Different pathogens were detected; *Pseudomonas aeruginosa* 48.5%, *Staph saprophyticus* 12.7%, *Staph epidermidis* 12.0%, and 6.0% for each of *Staph aureus* and *Shigella flexneri*. Percent of resistance to penicillin, novobiocin, streptomycin, gentamicin, tobramycin, and ceftazidime were 79.0%, 77.2%, 68.4%, 54.4%, 37.7%, and 17.5% respectively. All the isolates were sensitive to ciprofloxacin except *Shigella flexneri* showed intermediate resistance. *Pseudomonas,* the main isolated pathogen was mainly susceptible to ciprofloxacin and ceftazidime while resistant to penicillin, novobiocin, gentamicin, and streptomycin. In conclusion, it is compulsory to apply strict aseptic techniques in each procedure throughout the IVM procedure. Using ciprofloxacin in the culture media provides better inhibition than penicillin and streptomycin.

Keywords: Contamination, *Pseudomonas*, Antimicrobial-Resistance, Oocytes, In-vitro Maturation (IVM).

*Corresponding author: Shimaa T. Omara, Email: shimaaomara2112@gmail.com, Tel.: 01004022616
ORCID: 0000-0002-3521-9665
(Received 18/05/2023, accepted 29/07/2023)
DOI: 10.21608/EJVS.2023.211815.1503

Introduction

In-vitro maturation (IVM) of the bovine and camel oocytes is one of the main vital steps in the maximization of in-vitro embryo production (IVEP). Throughout the IVM, the oocytes procure the intrinsic capacity for step-by-step growth until witching on the embryonic genome [1]. However, the IVEP culture system is not a sterile system and microbial contamination of this system is a critical issue for IVEP laboratories. Microorganisms are famous to flourish in all types of circumstances and can be smoothly transported to IVEP laboratories colonizing the culture dishes of oocytes and embryos at various stages of the IVEP process [2]. If the oocytes or the embryo's culture dishes were contaminated with bacteria, the embryos may look dark and atretic and of poor quality or non-viable [3]. Generally, contamination of gametes can result in contamination of the embryo culture media resulting in damage to cultured oocytes and embryos [4]. Even if contaminated embryos are transferred to the micro-environment of the uterus, it will alter the local uterine microbiota and compromise implantation and survival during pregnancy [2].

With the inadequacy of standardization for sterility requisite standards and checking exercises in the IVEP laboratories as well as with the high incidence of microorganisms in the environment and gametes, it is logical to assume that microbial contamination in the IVEP laboratories can't be underestimated even with the strictest IVEP laboratories. The occurrence of contamination in the IVEP culture technique sounds like it is the rule rather than the deviation. Occasionally washing steps may dilute microbial colonies preventing them from being visible in flocculation shape. Sometimes, cultural requirements are not advantageous for colony formation. Such, however, does not mean that microorganisms are not present particularly when the potential of contamination via outside sources is considered. The primary external sources of contamination during in-vitro procedures are the personnel, environmental air, and contaminated materials, reagents, devices, and equipment. While the main internal source of contamination during in-vitro procedures is the follicular fluid [2]. On the other hand, although antibiotics are routinely added to the cultures to prevent bacterial contamination, these antibiotics might initiate the development of antibiotic-resistant bacterial strains with late-growing properties [5].

The limited number of research articles including information about the prevalence of contamination events in the IVEP laboratories makes the definite evaluation of the prevalence of microbial contaminations challenging. The current study aimed to determine the prevalence of bacterial contamination during the IVM procedure in an Egyptian veterinary IVF laboratory and to associate them with specific clinical outcomes with suggestions of what might be done to decrease the influence of such an event.

Material and Methods

In-Vitro Maturation (IVM)

Ovaries collection

Bovine and Camel's ovaries were collected from commercial abattoirs located in Giza and Minufiya governates, Egypt. This study was approved by National Research Centre Medical Research Ethics Committee (Ethical approval number: 16233). The ovaries were separated shortly after the slaughter of animals, dissected away from the surrounding tissues, and maintained in a thermos flask containing the transport medium (sterile normal saline supplemented with 100.000 IU/ml penicillin, 100 mg/ml streptomycin at 30-35^0 C within 1-2 hrs.). In the laboratory, the ovaries were washed three times with warm normal saline to remove adhering blood. The non-ovarian tissues were dissected away from the collected ovaries [6].

Collection and processing of oocytes

The oocytes were collected using the aspiration method. The aspiration medium used for the preparation of oocytes was modified phosphate buffer saline (M-PBS) supplemented with Na pyruvate (0.036 gm/ml), glucose (2.25 gm/ml), 10% FCS, heparin, and gentamycin. The follicular fluid was left to settle down for up to 30 minutes in a water bath (at 37^0 C), then about two-thirds of the supernatants were discarded and the residue was diluted with M-PBS solution, and then transferred into an 85.0 mm searching dish [7]. This procedure was repeated until all the ovaries were processed. The collected oocytes were washed three times with aspiration medium (M-PBS + 3% BSA). The oocytes were picked up with a glass Pasteur pipette of suitable diameters, under a binocular microscope. The oocytes were counted and classified according to their quality into three categories according to [8]. The categories of oocyte quality were as compact cumulus oocyte (COC'S), partially denuded oocyte (POC), and denuded oocyte (DCO).

Only COCs with identical cytoplasm and dense cumulus cells were used [9]. Each 5 - 10 oocytes were placed into a 4 wells petri dish containing 50.0µl droplets of the maturation medium and covered with paraffin oil (Sigma, USA) which was previously filtered through 0.22 µm filter paper. And then transferred to 100 ml glass bottles and autoclaved. Media and mineral oil were pre-exposed to the culture conditions for at least two hours before starting incubation to allow equilibrium of temperature and gases. Oocytes were cultured at 39˚C under 5 % CO_2 and 90 % relative humidity for 24 hours for bovine oocytes and 36 hours for camel oocytes in a CO_2 incubator [1]. Maturation was assessed by the determination of cumulus cell mass expansion or by the presence of the first polar body. Any turbid culture plates not calculated in the maturation rate and suspected of having microbial contamination were transported to the Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Cairo, Egypt where they were analyzed for the presence of microorganisms within one hour of collection.

Effect of leptin hormone addition on bovine oocytes maturation rate.

Different concentrations of leptin hormone (0.0, 50.0, 75.0, and 100.0 ng/ml) were added into three maturation media (TCM_{199}, $Hams_{F10}$, and Ferticult). All media were sterilized by passing through Millipore filter membrane (0.22µm) fitted on a 10 ml disposable syringe [10]. The experimental groups were classified as follows: Group 1**.** (TCM_{199}, $Hams_{F10}$, and Ferticult) without leptin (as control). Group2. (TCM_{199}, $Hams_{F10}$, and Ferticult) supplemented with Leptin (50.0ng/ml). Group3. (TCM_{199}, $Hams_{F10}$, and Ferticult) supplemented with Leptin (75.0ng/ml)**.** Group 4. (TCM_{199}, $Hams_{F10}$, and Ferticult) supplemented with Leptin (100.0ng/ml)**.** The maturation media was prepared with specific pH (7.4) and osmolarity about 300 mOsmol/Kg, and filtered using a 0.22 mm-millipore filter. A sterile petri dish was used to hold 200ml of the prepared maturation medium, which was covered with sterile mineral oil. Before placing oocytes in the culture dish, the Petri dishes were incubated in a Co2 incubator at 38.5˚C and high humidity (90.0 - 95.0%) for one hour for equilibration. Oocytes were cultured for 24 hours at 38.5˚C under 5% Co_2 in air and 90-95% relative humidity [11].

The effect of the type of media on the maturation rate of camel oocytes.

The maturation media (TCM 199 and HamsF12) used were supplemented with different additives such as Pregnant mare serum gonadotropin (PMSG) and Estradiol (E2) hormones, antibiotics (Gentamycin sulfate), and antioxidants (βME, RJ, Vit C, Se, and MEL) and enriched with heat-inactivated 10% serum (FCS or FDCS). Maturation was assessed as discussed previously.

Microbiologic Examination

Examined Samples for Bacterial Contamination

A total of 134 samples of the IVM procedure including 10 normal salines after handling (used for washing ovaries), 9 cystic ovaries, 10 large follicles, 8 ovarian surfaces, 10 follicular aspirates, 11 phosphate buffer salines (PBS), 25 tissue cultures (in test tubes), 36 inoculated tissue cultures with oocytes (in Petri dishes), and 15 paraffin oil were examined for the presence of bacterial contamination which frequently leads to experimental failure.

Preparation of sample

The samples were collected under aseptic conditions in sterile containers and transported to the Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Cairo, Egypt where they were nalysed for the presence of microorganisms within one hour of collection. 0.5 ml of normal salines after handling (used for washing ovaries), follicular aspirates, phosphate buffer salines (PBS), tissue cultures (in test tubes), inoculated tissue cultures with oocytes (in Petri dishes), and paraffin oil were inoculated using a sterile syringe into sterile tubes containing 4.5 ml Brain-Heart Infusion broth (CM1135, Oxoid, UK). Swabs of ovarian surfaces were inoculated into sterile tubes containing 5ml Brain-Heart Infusion broth. The cystic ovaries and large follicles were homogenized in a mortar and 0.5 gm of each sample was inoculated into 4.5 ml Brain-Heart Infusion broth. The broth was then incubated for 24 h at 37˚C for enrichment.

Isolation and Identification of Bacterial Pathogens

For the isolation and identification of *Pseudomonas*, samples were grown on *Pseudomonas* agar base media (CM0559, Oxoid, UK) and were handled according to [12]. *Staphylococcus* species isolation was carried out using International Standards Organization [13]. *Staph* identification was confirmed through

API-Staph identification Kit (20500, bio-Merieux, France) according to the manufacturer's instructions. For the isolation and identification of *Campylobacter,* samples were manipulated according to [14]. *Enterobacteriaceae* were isolated using Violet Red Bile Glucose agar (VRBGA) (CM1082, Oxoid, UK) and incubation at 37℃ for 24 h according to [15]. Typical colonies of the family *Enterobacteriaceae* were confirmed by the API system (20701, Biomerieux SA, Marcy l'Etoile, France) according to the manufacturer's instructions.

Antimicrobial Susceptibility Testing of the Recovered Bacterial Isolates

The isolates were tested for their antimicrobial resistance profiles using the Kirby Bauer disk diffusion method based on the National Committee for Clinical Laboratory Standards [16]. This experiment was done using Mueller-Hinton agar (CM0337, Oxoid, UK) and the following antimicrobial-impregnated disks (Oxoid, UK), including penicillin G (10 IU), streptomycin (25 µg), gentamicin (30 µg), novobiocin (30 µg), ciprofloxacin (5 µg), tobramycin (10 µg), and ceftazidime (30 µg). After inoculation of the antimicrobial-impregnated disks in the cultured Mueller-Hinton agar plates, the plates were then incubated overnight at 37°C and the zone sizes of inhibition were measured in millimeters and interpreted according to the National Committee for Clinical Laboratory Standards guidelines.

Statistical Analysis

Data were subjected to statistical analysis including the calculation of mean (M) ± standard error (SE), one-way ANOVA, and two-way ANOVA at a confidence limit of 95% ($p<0.05$). Statistical analyses were conducted according to the method described in 1971 [17] using the practicing statistical analysis program SPSS for Windows, version 16 (2007) Chicago: SPSS Inc [18]. Duncan's multiple range tests were used for testing pairs of means for comparison at a probability of 5% [19,20].

Results

Effect of leptin hormone addition on bovine oocytes maturation rate

The data presented in Table 1 demonstrates that the addition of leptin hormone to the maturation medium significantly increased the percentage of bovine oocytes that reached Germinal Vesicle (GV), Metaphase I (MI), Metaphase II (MII), and Degenerated stages during in-vitro maturation. However, there was no effect on the percentage of oocytes reaching Germinal Vesicles Breakdown (GVBD). The results showed that adding 75.0 ng/ml of leptin to the maturation medium had the highest maturation rate, with significantly higher percentages of oocytes reaching MII and lower percentages at GV, GVBD, MI, and degenerated stages compared to free leptin medium. Similarly, adding 50.0, 75.0, and 100.0 ng/ml of leptin to the maturation media also resulted in significantly

TABLE 1. Effect of leptin hormone supplementation to in-vitro maturation media on the maturation rate of bovine oocytes

Medium	Leptin Concentration (ng/ml)	Oocytes (No.)	Criteria of maturation rate										
			Germinal vesicle		Germinal vesicle breakdown		Metaphase-I		Metaphase-II		Total Maturation Rate%	Degeneration Rate	
			No.	%	No.	%	No.	%	No.	%		No.	%
TCM_{199}	Control	131	15	11.45[a]	16	12.21[a]	18	13.74[a]	53	40.46[b]	77.86	29	22.14[a]
	50.0	86	10	11.63[a]	11	12.79[a]	12	13.95[a]	40	46.51[b]	84.88	13	15.12[b]
	75.0	108	9	8.33[b]	11	10.19[a]	14	12.96[a]	63	58.33[a]	89.81	11	10.19[c]
	100.0	103	10	9.71[a]	12	11.65[a]	12	11.65[a]	54	52.43[a]	85.44	15	14.56[b]
$Hams_{F10}$	Control	173	21	12.14[a]	24	13.87[a]	26	15.03[a]	78	45.09[b]	86.13	24	13.87[a]
	50.0	90	11	12.22[a]	12	13.33[a]	15	16.67[a]	42	46.67[b]	88.89	10	11.11[a]
	75.0	98	12	12.24[a]	13	13.27[a]	15	15.31[a]	45	45.92[b]	86.73	13	13.27[a]
	100.0	106	11	10.38[a]	14	13.21[a]	14	13.21[a]	53	50.00[a]	86.79	14	13.21[a]
Ferticult	Control	112	14	12.30[a]	15	13.39[b]	17	15.18[a]	56	50.00[b]	91.07	10	8.93[b]
	50.0	118	14	11.86[a]	14	11.86[b]	16	13.56[a]	66	55.93[a]	93.22	8	6.78[b]
	75.0	120	10	8.33[b]	12	10.00[b]	13	10.83[a]	69	57.50[a]	86.67	16	13.33[a]
	100.0	109	10	9.17[a]	19	17.93[a]	14	12.84[a]	51	46.79[c]	86.24	15	13.76[a]

a – c: Values with different superscriptis within a row are significantly different ($P<0.05$)

TCM_{199}: Tissue- Culture Medium $Hams_{F10}$: Hams- Nutrient Medium

higher percentages of oocytes at MII and lower percentages of degenerated oocytes compared to free leptin medium with different media types (TCM199, Hams F10, and Ferticult).

Effect of type of maturation media on the maturation rate of contaminated bovine oocytes

The results presented in Table 2 and Fig. 1 show no significant effect of any type of maturation media on the maturation and degeneration rates of contaminated bovine oocytes. However, the highest degeneration rate was observed in Hams F10 media and the lowest was observed in Ferticult.

Effect of type of maturation media on the maturation rate of contaminated camel oocytes

The results presented in Table 3 and Fig. 2 show that TCM-199 has a highly significant effect (75.09±1.86%, *P<0.05*) on the maturation rate of contaminated camel oocytes compared to Ham`s F-12 (66.64±2.00%). Furthermore, The TCM 199 had less degeneration rate (24.91± 1.86%) than Ham`s F-12 (33.36%, P<0.05).

TABLE 2. Effect of type of maturation media on the maturation rate of contaminated bovine oocytes.

Type of medium	Oocyte (No.)	Maturation Rate%	Degeneration Rate%
TCM_{199}	400	78.75	19.75
$Hams_{F10}$	450	77.33	20.88
Ferticult	440	81.81	18.40

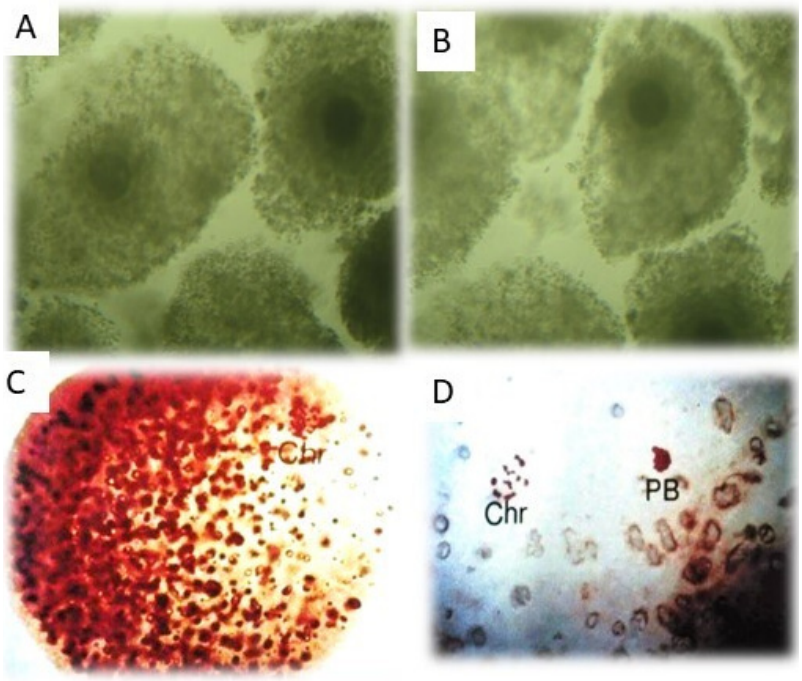

Fig. 1. A,B: The morphology of bovine cumulus-oocyte complexes under the inverted microscope (COC, 40x) after collected from an ovary, with a dense cumulus cell layer, to be cultured *in vitro* in maturation media *(IVM*) for 24 h. C: Metaphase I of bovine oocyte, the nuclear membrane has been broken-down and chromosomes (Chr.) can be seen clearly D: Metaphase II of bovine oocytes, the first polar body (PB) is present, and chromosomes (Chr.) are identified in the cytoplasm.

TABLE 3. Effect of type of maturation media on the maturation rate of contaminated camel oocytes.

Type of Media	Oocyte (No.)	Maturation Rate%	Degeneration Rate%
TCM- 199	338	75.14[b]	22.30[b]
Ham`s F-12	358	66.75[a]	27.65[a]

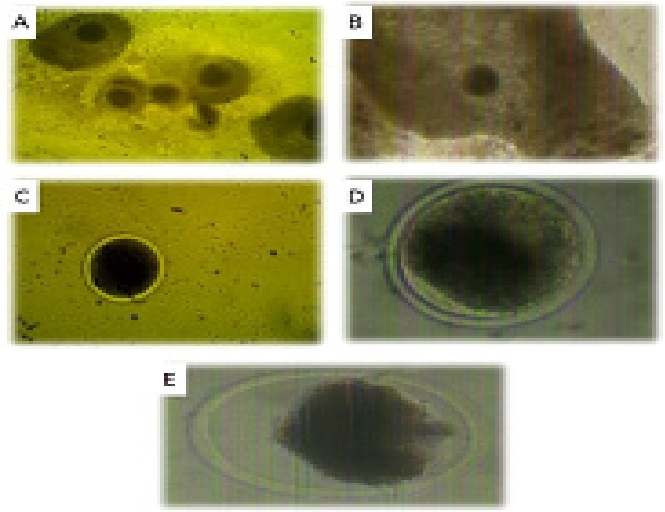

Fig. 2. A-B. mature camel oocyte surrounded by expanded cumulus cell (40x). C-D: matured oocytes showed extrusion of the first polar body above (40x) and down (100x). E: degenerated camel oocyte (100x).

Isolation and Identification of Bacterial Pathogens

From the results presented in Table 4, out of the examined 134 samples used in the IVM procedure, 114 (85.1 %, 114/134) were positive for bacterial contamination. Different pathogens were detected; *Pseudomonas aeruginosa* 48.5% (65), *Staph saprophyticus* 12.7% (17), *Staph epidermidis* 12.0% (16), and 6.0% (8) for each of *Staph aureus* and *Shigella flexneri,* while *Campylobacter* species were not detected at all. All the isolated samples except the cystic ovaries and large follicles were contaminated with *Pseudomonas aeruginosa*. *Shigella flexneri* was only isolated from salines after handling. *Staph saprophyticus* was isolated from salines after handling and large follicles. *Staph. epidermidis* was isolated from salines after handling and large follicles. *Staph. aureus* was only isolated from the surface of the ovaries.

Antibiotic Resistance Profile of the Isolated Pathogens

Table 5 and Fig. 3 show the antimicrobial resistance features of the isolated pathogens from tested samples. Percent of resistance to penicillin, novobiocin, streptomycin, gentamicin, tobramycin, and ceftazidime were 79.0% (90), 77.2% (88), 68.4% (78), 54.4% (62), 37.7% (43), and 17.5% (20) respectively. All the isolated pathogens were sensitive to ciprofloxacin except *Shigella flexneri* showed intermediate resistance. *Pseudomonas,* the main isolated pathogen was mainly susceptible to ciprofloxacin 100% (65) and ceftazidime 96.9% (63) while resistant to penicillin100% (65), novobiocin 96.9% (63), gentamicin 95.4 % (62), and streptomycin 92.3% (60).

IVM after using specific antibiotics.

Tables 6 and 7 show the maturation rates of bovine and camel oocytes after treatment with penicillin (100.000 IU/ml) and streptomycin100) mg/ml), ciprofloxacin (5µg), and ceftazidime (30µg). Table 8 shows the maturation rates of dromedary she-camel oocytes in-vitro matured for 36 hrs with different antioxidants after treatment with penicillin (100.000 IU/ml) and streptomycin 100) mg/ml), ciprofloxacin (5µg), and ceftazidime (30µg). The results showed that the maturation rate of bovine, camel, and dromedary she-camel oocytes was higher in the case of using ciprofloxacin and ceftazidime-enriched media

TABLE 4. Isolation and identification of different pathogens from samples used in the IVM procedure

Examined Samples		Results of Pathogen Detection											
Types of Samples	No. of Examined Samples	*Pseudomonas aeruginosa*		*Shigella flexneri*		*Staph saprophyticus*		*Staph epidermidis*		*Staph aureus*		*Campylobacter*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Salines after handling	10	10	100.0	8	80.0	7	70.0	10	100	0	0.0	0	0.0
Cystic ovaries	9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Large follicles	10	0	0.0	0	0.0	10	100.0	6	60.0	0	0.0	0	0.0
Surface of ovaries	8	8	100.0	0	0.0	0	0.0	0	0.0	8	100.0	0	0.0
follicular aspirates	10	10	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
PBS	11	٧	63.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Paraffin	15	5	33.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Tissue culture test tube	25	9	36.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Tissue culture Petri dishes	36	16	44.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total Sample	134	65	48.5	8	6.0	17	12.7	16	12.0	8	6.0	0	0.0

TABLE 5. Antimicrobial resistance features of the isolated pathogens

Pathogen detected/ Antimicrobial	Pseudomonas aeruginosa (no=65)			Shigella flexneri (no=8)			Staph saprophyticus (no=17)			Staph epidermidis (no=16)			Staph aureus (no=8)			Total R (Within 114 pathogens)	
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	No.	%
Penicillin	0	0	65	0	0	8	8	0	9	8	0	8	8	0	0	90	79.0
Streptomycin	5	0	60	0	8	0	6	0	11	9	0	7	8	0	0	78	68.4
Gentamicin	0	3	62	8	0	0	15	2	0	16	0	0	8	0	0	62	54.4
Novobiocin	0	2	63	0	0	8	0	0	17	16	0	0	8	0	0	88	77.2
Ciprofloxacin	65	0	0	0	8	0	17	0	0	16	0	0	8	0	0	0	0.0
Tobramycin	8	14	43	8	0	0	17	0	0	16	0	0	8	0	0	43	37.7
Ceftazidime	63	2	0	8	0	0	3	10	4	0	0	16	8	0	0	20	17.5

S= sensitive, **I**= intermediate, **R**=resistance

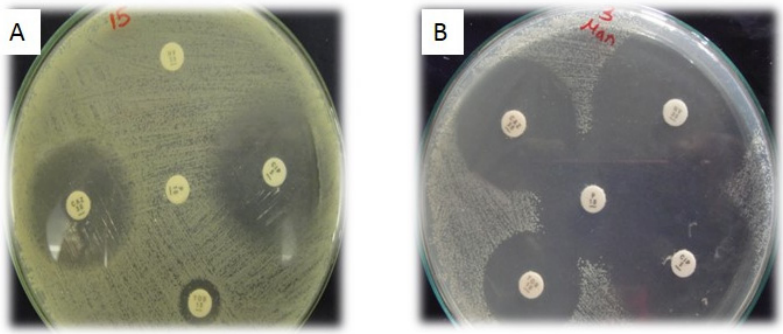

Fig. 3. Antimicrobial susceptibility patterns of (A) *Pseudomonas* and (B) *Staphylococcus aureus*

TABLE 6. Effect of different antibiotics on in-vitro maturation rate of bovine oocytes cultured in different media

Type of medium	Oocyte (No.)	Penicillin/ Streptomycin		Ciprofloxacin		Ceftazidime	
		M. %	D.%	M.%	D.%	M.%	D.%
TCM_{199}	120	76.66[b]	19.16[b]	85.83[a]	10.83[a]	84.16[a]	11.66[a]
$Hams_{F10}$	144	78.56[b]	17.36[a]	88.19[a]	9.02[a]	86.80[a]	10.41[a]
Ferticult	132	82.57[b]	12.87[b]	90.90[a]	6.06[a]	92.42[a]	4.54[a]

M. %: Maturation rate, D. %: Degeneration rate

TABLE 7. Effect of different antibiotics on in-vitro maturation rate of camel oocytes cultured in different media

Type of medium	Oocyte (No.)	Penicillin/ Streptomycin		Ciprofloxacin		Ceftazidime	
		M.%	**D%**	**M%**	**D%**	**M%**	**D%**
TCM_{199}	120	74.16[b]	20.83[b]	84.16a	9.16a	81.66a	12.50a
$Hams_{F-12}$	144	69.28[b]	25.71[b]	88.19a	8.33a	86.80a	9.02a

M. %: Maturation rate, D. %: Degeneration rate

TABLE 8. Effect of different antibiotics on the maturation rate of dromedary she-camel oocytes in-vitro matured for 36 hrs with different antioxidants.

Groups	Oocytes (NO.)	Penicillin/ streptomycin	Ciprofloxacin	Ceftazidime
Control	110	68.68 ± 3.89%	73.75± 3.34%	71.75± 4.21%
βME	134	72.18± 3.86%	75.69±2.45%	77.69±2.61%
RJ	104	67.88 ± 4.57%	79.12±3.76%	80.12±4.51%
Vit. C	142	72.30 ± 3.15%	82±2.41%	81±3.89%
Se	130	72.78 ± 3.19%	80.22±4.64%	79.22±3.67%
MEL	122	71.39 ± 2.85%	75.61±2.12%	79.61±3.42%

than using penicillin streptomycin-enriched media irrespective of the type of the culture media. The results of microbiological testing of the examined samples revealed that the best drug of choice to prevent contamination of the IVM plates is ciprofloxacin (5µg) and ceftazidime (30µg) and recommended to be used instead of penicillin/ streptomycin.

Discussion

Bovine oocyte maturation is a complex process that involves various factors, including hormonal regulation, cellular signaling pathways, and environmental factors [21]. The results of this study demonstrated that the supplementation of leptin hormone to the maturation media improves the maturation rate of bovine oocytes. Leptin is a hormone that plays a vital role in regulating energy balance and metabolism. It is also involved in the regulation of reproductive functions, including oocyte maturation. Several studies have investigated the effect of leptin addition on the maturation of bovine oocytes. A previous study found that adding leptin to the maturation

medium significantly improved the maturation rate and quality of bovine oocytes. The study showed that leptin supplementation increased the percentage of oocytes that reached the metaphase II (MII) stage, which is a critical stage for fertilization and embryonic development. The authors suggested that leptin may enhance oocyte maturation by promoting cumulus expansion and increasing levels of cyclic adenosine monophosphate (cAMP), which is essential for meiotic resumption [22]. Another study investigated the effect of different concentrations of leptin on bovine oocyte maturation. The results showed that low concentrations of leptin (0.1-1 ng/mL) significantly improved oocyte maturation rates compared to control groups, while higher concentrations (10-100 ng/mL) had no significant effect or even inhibited oocyte maturation. The authors suggested that optimal concentrations of leptin may be necessary for promoting bovine oocyte maturation [23]. In contrast, some studies have reported no significant effect of leptin on bovine oocyte maturation. For example, a previous study found that adding leptin to the maturation medium did not improve the rate or quality of bovine oocyte maturation [24]. This inconsistency may be attributed to the different doses used, while there are conflicting results regarding the effect of leptin addition on bovine oocyte maturation. Several studies suggest that optimal concentrations of leptin may improve oocyte quality and increase the percentage of oocytes reaching MII stage.

On the other hand, one of the environmental factors that can affect oocyte maturation is the microbial contamination. Microbial contamination during oocyte maturation can occur during various stages of oocyte handling and culturing, including oocyte collection, washing, and in-vitro maturation [21]. The occurrence of microbial contamination during the IVM procedure is considered a pervasive problem that should be carefully analyzed to control it. However, currently, there is a lack of detailed definitive investigations on the incidence of bacterial contamination in the IVEP laboratories especially those who are working in the veterinary sector. In addition, precise information regarding the possible influence of bacterial contamination on the IVM procedure is also lacking [2]. Regarding the previously mentioned issues, the samples used in the IVM procedure were examined by standard microbiologic examination. In the current study, 114 (85.1 %, 114/134) samples were positive for bacterial contamination. Different pathogens were detected; *Pseudomonas aeruginosa* 48.5%, *Staph saprophyticus* 12.7%, *Staph epidermidis* 12.0%, and 6.0% for each of *Staph aureus* and *Shigella flexneri*, while *Campylobacter* species were not detected at all. The presence of these bacterial genera in samples used in the IVM procedure in the veterinary sector was not previously published. In addition, as only very few research articles dealing with microbial contamination in the IVEP laboratories in the veterinary sector are available compared to the high record of research articles dealing with this topic in humans [4,25], a comprehensive comparison of data cannot be conducted. The high prevalence of microorganisms detected in the current study may be due to the precise and careful method by which culture dishes are checked and maybe also due to improper sterilization and standardization practices during working in the IVEP laboratory. Despite the high incidence of contamination detected in the current study, there are several explanations for the low number of research articles dealing with contamination in the IVEP laboratories as reported previously. Firstly, one cannot suppose that just considering one does not see any visible microbial contamination in the culture media, that contamination is not present, or that they do not affect IVF outcomes. Generally, every laboratory occasionally deals with infections. Secondly, an association of facts may result in a low incidence of visible contamination of IVM culture media. This might involve for example the slow growth rate of bacteria, low concentrations of bacteria, particularly after the plenty dilutions of media that occur throughout handling, the presence of antibiotics, and the culture environments which may be suboptimal for the bacterial growth [3]. Even if bacteria do not produce any visible signs such as flocculent or necrosis on the embryos or eggs, it could affect the embryo quality. Further, the incidence of contamination is independent of whether oocytes and embryos are managed on the bench in a low-traffic area or handled only in biosafety cabinets [25]. Indeed, embryologists have the problematic mission of trying to keep up a sterile environment and at the main time aiming to protect cultures from unfavorable environments. Some of the methods adopted to maintain the environment aseptically may exacerbate other issues resulting in lower embryo viability. For example, the inaccurate use of hoods might raise the possibility of contamination if there are non-sterile subjects in the biosafety cabinet intervening

in the flow of sterile air causing that contaminated air to move through sterile dishes or drops [3]. On the other hand, as incubation temperature is a governing factor for bacterial growth, incubation of IVF culture media at 37℃ could influence bacterial growth and activity. Recently, bacterial contamination by *Staphylococcus* spp., *E. coli*, and *Streptococcus* species was detected in IVF culture media [2]. In addition, a high level of bacterial contamination in 15 IVF cycles was recorded out of 30 examined cycles. The most common bacterial species detected were Mycoplasma hominis, *Staphylococcus epidermidis,* and Diptheroids [3]. Another study revealed that the most commonly identified species in IVF were *E. coli*, and gram-negative cocci [25]. A previous study has indicated that coliform bacteria, including *E. coli* and other bacteria, belonging to *Alphaproteobacteria* were found in higher concentrations in IVF culture media. If the embryo culture dishes are contaminated with bacteria like *Alphaproteobacteria* and *Enterobacteriaceae*, the quality of the developing embryos will be poor [4].

In the current study, the most common sources of bacterial contamination in the IVM procedure were the salines, the ovaries, large follicles, follicular aspirates, PBS, tissue culture test tube, and paraffin. As previously reported, the most common sources of bacterial contamination in the IVM culture were follicular aspirates, semen, and improper sterile technique [3]. A previous study has recorded that out of the examined 144 follicle aspirates, 143 were contaminated by different pathogens. Another survey on bacterial contamination through several IVF laboratories revealed that one of the most common sources of contamination was the improper sterile technique (23%). In another survey, technician contamination and oil were the most recorded sources of contamination [3]. The contamination sources are either from the outside or the inside. The outside air contains 10 to 100 CFUs /m^2 while the inside air contains 10 times CFUs more than the outside air. Ceilings and walls are hardly ever seriously contaminated and usually contain 2-5 colonies per 25 cm^2. Floors are considerably contaminated with 380 colonies per 25 cm^2. Most of this contamination takes the form of skin flora from the occupants. The environment that encompasses our bodies is full of microorganisms [3]. Our skin is colonized with plaques of *Staphylococcus epidermidis*, *S. aureus*, *Acinetobacter*, *Klebsiella, E. coli,* and other enterobacters. Every month humans get a new dermis as 30,000 to 40,000 skin cells fall off every minute. Human skin is often the source of the microbes found inside the laboratories as these shedding skin cells are responsible for the dust that is swept up in the laboratory [3].

The presence of microorganisms in the culture medium can lead to changes in the microenvironment of the oocyte and affect its developmental competence. In the current study, the percentage of degenerated bovine oocytes was increased in contaminated culture medium while the percentage of matured oocytes was decreased. Several studies have investigated the effect of microbial contamination on bovine oocyte maturation. One study found that bacterial contamination of the IVM culture medium significantly reduced the percentage of oocytes that reached the metaphase II (MII) stage compared to control groups that have no bacterial contamination [21]. Another study reported that the exposure to endotoxins produced by *Escherichia coli* during the IVM procedure reduced the proportion of MII oocytes and increased abnormal spindle formation [26]. The mechanism by which microbial contamination affects oocyte maturation is not fully understood. However, it is thought that bacterial toxins or metabolites may interfere with cellular signaling pathways involved in meiotic progression or disrupt the balance between oxidative stress and antioxidant defense mechanisms [27]. Cultures in which bacteria are grown can contain many toxic substances for example endotoxins, alpha-hemolysin, peptidoglycans, Shiga-like toxins, lipopolysaccharides, and peptidoglycans. Endotoxin, the lipopolysaccharide portion of the cell wall of gram-negative bacteria, can cause fragmentation and blebbing which influences the embryo culture and reduces the pregnancy rates. Alpha-hemolysin, a calcium-dependent cytolytic toxin secreted by some bacteria could have toxic effects on the cultured cells. It can incorporate itself into the cell membrane of the cultured cells forming pores that allow a high-speed outlet of potassium and an intake of sucrose, mannitol, and calcium, which by osmotic lysis leads to cell destruction [3].

Camelid reproduction is an important aspect of the livestock industry in arid and semi-arid regions. However, the maturation rate of camel oocytes can be affected by various factors, including microbial contamination. Microbial contamination can occur during the collection and

handling of camel oocytes, which can lead to a decrease in their quality and viability. In the current study, the contamination of the contaminated culture medium with microorganisms increased the degenerative rate of camel oocytes and reduced the percentage of camel oocyte maturation rate. In a previous study, *Escherichia coli* was isolated from the uteri of camels [28]. Another recent study found that *Staphylococcus aureus* contamination resulted in a decrease in the maturation rate of the oocytes [29]. The mechanism by which microbial contamination affects the maturation rate of camel oocytes is not fully understood. However, it is believed that bacterial toxins and metabolites can interfere with the signaling pathways involved in oocyte maturation. Additionally, bacterial contamination can lead to oxidative stress, which can also affect the quality and viability of oocytes.

To prevent microbial contamination during the collection and handling of bovine and camel oocytes, various measures have been proposed. These include, for example, washing hands thoroughly before oocyte handling, using sterile techniques during the collection and handling of oocytes, using sterile equipment and media, and optimizing culture conditions to promote a healthy microenvironment for the oocyte [29,30]. Simple cleaning can decrease the burden of bacteria by 80% and the use of a disinfectant can decrease it to 95%. Additionally, the use of a high-efficiency biosafety cabinet with a positive airflow can make sure that the cleanest air flows into the laboratory and the impure air is unable to leak into the laboratory. Even if by chance bacteria reach the culture media, almost all the media used in the IVF procedure comprises antibiotics to inhibit bacterial growth [3].

The use of antibiotics in assisted reproductive technologies (ART) has been a topic of interest for many years. Antibiotics are commonly used to prevent bacterial contamination during in-vitro maturation (IVM) of oocytes. Penicillin and streptomycin are the most common antibiotics used to prevent microbial contamination in the IVM culture media, effective against broad-spectrum gram-positive and gram-negative bacteria [4]. However, the result of the current study showed that the use of penicillin and streptomycin in the IVM culture media did not prevent microbial contamination. In the current study, the percent of resistance to penicillin and streptomycin were 79.0% and 68.4% respectively. The same to the current result, previous studies have recorded that although using penicillin and streptomycin, contamination occurred in the IVM culture media [5]. A previous study recorded that in 91% of bacterial contamination in the IVF procedure, the bacteria were resistant to both penicillin and streptomycin [3]. Recently, an increasing number of penicillin and streptomycin-resistant bacterial strains in the IVM culture media has been noticed [4]. 4.8% of bacterial contamination was outlined in a study in Brazilian IVF laboratories in spite of following hygienic steps and using antibiotics in the culture media. This is most probably due to bacterial strains resistant to the used antibiotics [2]. In 2005, due to the very short half-life of penicillin at 37°C (less than a couple of hours), penicillin has been displaced by a stable form of the aminoglycoside, gentamicin which exhibits no incidences of bacterial contamination [3]. Gentamicin is a broad-spectrum bactericidal antibiotic of the aminoglycoside group that is effective against Gram-negative and Gram-positive aerobic bacteria. Gentamicin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This leads to interference with the initiation complex and misreading of mRNA so that incorrect amino acids are inserted into the polypeptide leading to toxic or non-functional peptides and the breakup of polysomes into non-functional monosomes. Though the counts of *Enterobacteriaceae* reduced with gentamicin therapy, however, some bacterial species (*Phyllobacterium, Methylobacterium*) belonging to *Alphaproteobacteria* classes increased, as these bacterial species are gentamicin resistant [4]. However, in the current study, the isolated pathogens showed 54.4% resistance against gentamicin. The same to the current result, previous studies have recorded that using gentamicin provides limited inhibition to huge numbers of bacteria. Additionally, it was illustrated that aminoglycosides possess toxic effects on sperm motility [4]. On the other hand, the administration of clavulanic acid and amoxicillin before embryo transfer did not impact clinical pregnancy proportion [4]. Indeed, the effect of different antibiotics on the maturation rate of oocytes is not well understood. In the present study, the results of microbiological testing of the examined samples revealed that the best drug of choice to prevent microbial contamination of the IVM plates is ciprofloxacin (5µg) and ceftazidime (30µg) and recommended to be used instead of penicillin/streptomycin which should be taken into account during the IVM procedure

in the future. Furthermore, the maturation rate of bovine, camel, and dromedary she-camel oocytes in the current study was higher in the case of using ciprofloxacin and ceftazidime-enriched media than using penicillin streptomycin-enriched media irrespective of the type of the culture media. A previous study investigated the effect of three different antibiotics, namely penicillin, streptomycin, and gentamicin, on the contamination of oocytes during IVM. The results showed that the addition of penicillin and streptomycin had no significant effect on the contamination rate compared to the control group. However, the addition of gentamicin significantly decreased the contamination rate [31]. On the contrary, another study showed that the penicillin and streptomycin antibiotics enriched culture medium is sufficient to prevent microbial contamination which affects the maturation rate of oocytes [32]. These findings suggest that not all antibiotics have a positive effect on oocyte maturation during IVM. In fact, some antibiotics may have a negative impact on this process. Therefore, it is important to carefully select and evaluate antibiotics used in IVEP laboratories to ensure optimal outcomes. The development of a worldwide database with information about the microbiological monitoring of every IVM cycle in the veterinary sector and annual reports of contamination cases could be useful to better understand and manage the impact of worldwide microbial contamination on IVM and to develop a healthy culture system for immature oocytes.

Conclusion

In conclusion, the IVM procedure does not occur in a sterile environment. Microbial contamination by antibiotic-resistance *P. aeruginosa, S. flexneri, S. saprophyticus, S. epidermidis, and S. aureus* was recorded during the IVM procedure. Using ciprofloxacin and ceftazidime as antibiotics in the IVM culture media may provide better inhibition than penicillin/streptomycin. To reduce or eliminate the potential of introducing bacteria into the IVF laboratory, it is compulsory to apply strict aseptic techniques in each procedure throughout the IVM procedure. The exposure time of gametes to the outside environment should be kept at a minimum. Finally, the supplementation of leptin hormone to the maturation media can improve the maturation rate of bovine oocytes.

Acknowledgment

Not applicable.

Conflict of interest

There is no conflict of interest.

Funding statement

No funding was received for conducting this study.

References

1. Pioltine, E.M., Costa, C.B., Latorraca, L.B., Franchi, F.F., dos Santos, P.H., Mingoti, G.Z., Paula-Lopes, F.F. and Nogueira, M.F.G. Treatment of in vitro-Matured Bovine Oocytes with Tauroursodeoxycholic Acid Modulates the Oxidative Stress Signaling Pathway. *Front. Cell Dev. Biol.,* **9**, 623852 (2021).

2. Borges, E.D., Berteli, T.S., Reis, T.F., Silva, A.S. and Vireque, A.A. Microbial contamination in assisted reproductive technology: source, prevalence, and cost. *Journal of Assisted Reproduction and Genetics*, **37**, 53-61 (2020).

3. Pomeroy, K.O. Contamination of Human IVF Cultures by Microorganisms: A Review. *The Journal of Clinical Embryology,* **13(**4)5-10 (2014).

4. Štšepetova, J., Baranova, J., Simm, J., Parm, Ü., Rööp, T., Sokmann, S., Korrovits, P., Jaagura, M., Rosenstein, K., Salumets, A. and Mändar, R. The complex microbiome from native semen to embryo culture environment in human in vitro fertilization procedure. *Reproductive Biology and Endocrinology,* **18**, 3 (2020).

5. Campos, C.O., Bernuci, M.P., Vireque, A.A., Campos, J.R., Silva-de-S´a, M.F., Jamur, M.C. and Rosa-e-Silva, A.C.J.S. Preventing Microbial Contamination during Long-Term In Vitro Culture of Human Granulosa-Lutein Cells: An Ultrastructural Analysis (2012). *ISRN Obstetrics and Gynecology,* **2012**, Article ID 152781, 6 pages (2012).

6. Lee, E.S. and Fukui, Y. Effect of various growth factors in a defined culture medium on in vitro development of bovine embryos matured and fertilized in vitro. *Theriogenology,* **44**, 71-83 (1995).

7. Botigelli, R.C., Razza, E.M., Pioltine, E.M., Fontes, P.K., Schwarz, K.R.L. and Leal, C.L.V. Supplementing in vitro embryo production media by NPPC and sildenafil affect the cytoplasmic lipid content and gene expression of bovine cumulus-oocyte complexes and embryos. *Reprod. Biol.,* **18**, 66–75 (2018).

8. Kim, S.K. and Park, H.K. Studies on the in vitro maturation rate of bovine follicular oocytes. *Theriogenology,* **33**, 26-30 (1990).

9. Seneda, M.M., Esper, C.R., Garcia, J.M., De Oliveira, J.A. and Vantini, A.M. Relationship between follicle size and ultrasound-guided transvaginal oocyte recovery. *Anim. Reprod. Sci.,* **67**, 37-43 (2001).

10. Leibfried-Rutledge, M.L., Critser, E.S. and First, N.L. Fertilization potential of follicular oocytes classified by stage of cycle and size of follicle. *Theriogenology,* **23**, 753-759 (1985).

11. Wright, Jr. R.W. and Bodioli, K.R. Aspects of in vitro fertilization and embryo culture in domestic animals. *J. Anim. Sci.,* **53**, 701-729 (1981).

12. Ali, N.G., Ali, T.E., Aboyadak, I.M. and Elbakry, M.A. Controlling *Pseudomonas aeruginosa* infection in *Oreochromis niloticus* spawners by cefotaxime sodium. *Aquaculture,* **544**, 737107 (2021).

13. ISO (2021) ISO (International Standards Organization) 6888-1:2021 Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 1: Method using Baird-Parker agar medium (2021).

14. Omara, S.T., El Fadaly, H.A. and Barakat, A.M.A. Public Health Hazard of Zoonotic *Campylobacter jejuni* Reference to Egyptian Regional and Seasonal Variations. *Research Journal of Microbiology,* **10** (8), 343-354 (2015).

15. Craven, H., McAuley, C., Hannah, M., Duffy, L., Fegan, N. and Forsythe, S. Applicability of Enterobacteriaceae and coliforms tests as indicators for *Cronobacter* in milk powder factory environments. *Food Microbiology,* **94**, 103642 (2021).

16. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twenty-third informational supplement M100-S23. CLSI, Wayne, PA, USA, **33**, 1-199. (2013).

17. Armitage, P. Statistical methods in medical research. *Blackwell Scientific Publications,* (1971).

18. SPSS Inc. SPSS Statistics for Windows, Version 16.0. Chicago: SPSS Inc. (2007).

19. Duncan, D.B. Multiple range and multiple F tests. *Biometrics,* **11** (1), 1-42 (1955).

20. Steel, R.G. and Torrie, J.H. Principles and procedures of statistics: A biometrical approach (2nd ed.). *McGraw-Hill Book Company* (1980).

21. Rizos, D., Ward, F., Duffy, P., Boland, M.P. and Lonergan, P. Bacterial lipopolysaccharide disrupts in vitro oocyte maturation and subsequent embryo development in cattle. *Biology of Reproduction,* **66** (3), 614-619 (2002).

22. Khaki, A., Batavani, R., Najafi, G., Tahmasbian, H., Belbasi, A. and Mokarizadeh, A. Effect of Leptin on In Vitro Nuclear Maturation and Apoptosis of Buffalo (Bubalus bubalis) Oocyte. *Int. J. Fertil. Steril.*, **8** (1), 43-50 (2014).

23. Arias-Alvarez, M., Bermejo-Alvarez, P., Gutierrez-Adan, A., Rizos, D., Lorenzo, P.L. and Lonergan, P. Effect of leptin supplementation during in vitro oocyte maturation and embryo culture on bovine embryo development and gene expression patterns. *Theriogenology,* **75** (5), 887–896 (2011).

24. Córdova, B., Morató, R., de Frutos, C., Bermejo-Álvarez, P., Paramio, T., Gutiérrez-Adán, A. and Mogas, T. Effect of leptin during in vitro maturation of prepubertal calf oocytes: embryonic development and relative mRNA abundances of genes involved in apoptosis and oocyte competence. *Theriogenology,* **76** (9), 1706-1715 (2011).

25. Kastrop, P.M.M., de Graaf-Miltenburg, L.A.M., Gutknecht, D.R. and Weima, S.M. Microbial contamination of embryo cultures in an ART laboratory: sources and management. *Human Reproduction*, pp. 2243–2248 (2007).

26. Sugimura, S., Akai, T., Hashiyada, Y. and Aoki, F. Bacterial endotoxin (lipopolysaccharide) reduces the developmental competence of bovine oocytes in vitro. *Reproduction*, **129** (3), 373-380 (2005).

27. Kafi, M., Kohram, H., Zhandi, M., Sharafi, M. and Sadeghi-Sefidmazgi, A. The effect of bacterial endotoxin on bovine oocyte quality and maturation in vitro. *Veterinary Research Forum. An International Quarterly Journal*, **7** (1), 51-57 (2016).

28. Asadi, B., Seyedasgari, F., Tamai, A.T., Yarmohammadi, M., Ebadi, R., Kim, E. and Barin, A. Isolated Bacteria from the Uteri of Camels with Different Reproductive Backgrounds: A Study on Sampling Methodology, Prevalence, and Clinical Significance. *Vet. Sci.,* **10** (1), 39 (2023).

29. Zheng, T., Li, Q., Chen, N., Du, P. and Ye, H. Analysis of the clinical outcomes of microbial contamination caused by environmental contamination of the embryology laboratory during IVF-ET treatment cycles. *BMC Pregnancy Childbirth*, **23**, 190 (2023).

30. Cognie, Y., Chupin, D. and Poulin, N., Techniques and protocols for in vitro production of embryos. 3rd ed. Wallingford, UK: *CABI Publishing*, pp. 55-72 (2014).

31. Elsner, H.A., Hönck, H.H., Willmann, F., Kreienkamp, H.J. and Iglauer, F. Poor quality of oocytes from Xenopus laevis used in laboratory experiments: prevention by use of antiseptic surgical technique and antibiotic supplementation. *Comp. Med.,* **50** (2), 206-211 (2000).

32. Cottell, E., McMorrow, J., Lennon, B., Fawsy, M., Cafferkey, M. and Harrison, R.F. Microbial contamination in an in vitro fertilization-embryo transfer system. *Fertil Steril.,* **66** (5), 776–80 (1996).

التلوث بميكروبات مقاومة للمضادات الحيوية أثناء نضج بويضات الأبقار والإبل في المختبر: الأسباب والإدارة

شيماء توفيق عبدالله عماره[١]، احمد ناجى[٢]، مياده حسن[٣]، احمد فراج[٤] و عبد المحسن محمد همام[٢]

[١] قسم الميكروبيولوجيا والمناعه - المعهد القومي البيطري - المركز القومي للبحوث - القاهرة - مصر.
[٢] قسم التكاثر الحيوانى والتلقيح الاصطناعى - المعهد القومي البيطري - المركز القومي للبحوث - القاهرة - مصر.
[٣] قسم التلقيح الاصطناعى ونقل الأجنة - معهد بحوث التناسليات الحيوانية - مركز البحوث الزراعية - الجيزة- مصر.
[٤] قسم بحوث تربية الماشية - معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية- الجيزة- مصر.

لا يمكن التقليل من أهمية التلوث الميكروبي في مختبرات إنتاج الأجنة في المختبر (IVEPانتاج الاجنه فى المعمل) حتى مع أشد مختبرات IVEP صرامة. تهدف الدراسة الحالية إلى تحديد مدى انتشار التلوث البكتيري أثناء إجراء النضج في مختبرللتلقيح الاصطناعي البيطري وربطها بنتائج سريرية محددة مع اقتراحات لما يمكن القيام به لتقليل تأثير مثل هذا الحدث. شملت الدراسة ١٣٤ عينة تم استخدامها في إجراء IVMالنضج للبويضات فى المعمل وهم؛ المحلول الملحي العادي، والمبيض الكيسي، والبصيلات الكبيرة، وأسطح المبيض، والشفرات الجريبية، والملح العازل للفوسفات (PBS)، وزراعة الأنسجة، وزراعة الأنسجة الملقحة مع البويضات، وزيت البارافين. تم فحص العينات بحثًا عن وجود تلوث بكتيري باستخدام الاختبارات المورفولوجية والميكروبيولوجية والكيميائية الحيوية القياسية. تم اختبار مدى مقاومة المعزولات البكتيرية للمضادات الحيويه باستخدام طريقة انتشار قرص كيربي باور. كانت نسبة ٨٥,١٪ من العينات التي تم فحصها إيجابية للتلوث البكتيري. تم الكشف عن مجموعه مختلفه من البكتيريا ؛ 48.5 *Pseudomonas aeruginosa*٪، *Staph saprophyticus* 12.7٪، 12.0 *Staph epidermidis*٪، و ٦,٠٪ لكل من *Staph aureus* و *Shigella flexneri*. كانت نسبة مقاومة البنسلين والنوفوبيوسين والستربتومايسين والجنتاميسين والتوبراميسين والسيفتازيديم ٧٩,٠٪ و ٧٧,٢٪ و ٦٨,٤٪ و ٥٤,٤٪ و ٣٧,٧٪ و ١٧,٥٪ على التوالي. كانت جميع العزلات حساسة للسيبروفلوكساسين باستثناء *Shigella flexneri* أظهرت مقاومة متوسطة. *Pseudomonas*، العامل الممرض الرئيسي المعزول كان عرضة بشكل أساسي للسيبروفلوكساسين والسيفتازيديم بينما كان مقاومًا للبنسلين ونوفوبيوسين والجنتاميسين والستربتومايسين. في الختام، من الإلزامي تطبيق تقنيات التعقيم الصارمة في كل خطوه طوال إجراء IVM. يوفر استخدام السيبروفلوكساسين في الوسائط الثقافية تثبيطًا أفضل من البنسلين والستربتومايسين.

الكلمات الدالة : الاخصاب المجهري-الابقار- الجمال- التلوث-الاوساط الصناعية