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Effect of oviduct and follicular fluids on ram sperm capacitation and acrosome reaction in vitro

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ABSTRACT

The present study was designed to study the influence of different concentrations of oviduct fluid (OF) and follicular fluid (FF) on ram sperm capacitation and acrosome reaction in vitro. Forty semen samples were collected from three Barki rams throughout the period of study (10 weeks). Fresh semen was evaluated, layered under S-TALP (sperm Tyrode's albumin lactate pyruvate) medium and subjected to swim up techniques. Split fractions of semen were incubated in media enriched with different levels of OF (10, 20, 40, 50 and 75 μL/mL) or FF (10, 20, 40, 50, 100, 150 and 200 μL/mL). Best concentrations were compared and used to evaluate the ram sperm functions including progressive motility, hyperactivity and acrosome reaction. The present findings showed a significant increase in individual motility percentage (IM %) when ram spermatozoa were treated with 10 and 50 μL of oviduct fluids as compared to the other treatments after 1 h of incubation. Addition of 50 μL/mL of OF or FF had beneficial effect on sperm hyperactivity after 2 and 1 h incubation respectively. Furthermore, addition of 50 μL/mL oviduct fluid to ram sperm maintained significantly ($P < .05$) higher total acrosome reaction (AR %) after 3 h of incubations than those observed in other groups. In conclusion, treatment of ram spermatozoa with 50 μL/mL of OF or 40 μL/mL of FF for 3–4 h incubation respectively was considered the best level of oviduct or follicular fluid to be used for IVF.

1. Introduction

In vivo, eutherian mammalian spermatozoa stay a few hours in female genital tract, until they are able to fertilize oocytes [1]. Within the female genital tract, spermatozoa undergo several changes including biochemical, metabolic and structural changes. These events, were earlier termed as capacitation [2]. The biochemical changes in sperm plasma membrane include an increase in plasma membrane fluidity, increase in Ca^{2+} ions influx, induction of tyrosine phosphorylation and increase in sperm metabolism and expression of hyperactivity [3,4]. Capacitation process causes sperm to undergo a spontaneous acrosome reaction (AR). Physiological agonists are suggested to be inducer of AR including zona pellucida protein (ZP3), follicular fluid (FF) and oviduct fluid (OF). In previous reports, oviduct fluid derived from two sources: serum transudate and secretions of surrounding oviductal epithelium [4–6]. Numerous studies showed that oviductal secretions are crucial for enhancement of sperm motility, capacitation, acrosome reaction and sperm-zona attraction [7–9]. At the time of ovulation, follicular fluid components may pass into the oviduct or close to oocyte cumulus complex. Follicular fluid is a product derived from blood plasma constituents and the secretory activity of granulosa and thecal cells [10,11]. Follicular fluid has been shown to have several effects on sperm cells. Stimulation of sperm respiration by in vitro exposure to follicular fluid from homologous species has been reported in sheep [12], cattle [13,14] and buffalo [15]. Therefore the present study was designed to evaluate the effect of different levels of oviduct fluid (OF) and follicular fluid (FF) with different incubation times on ram sperm motility, hyperactivity (HA) and acrosome reaction (AR) in vitro.

2. Materials and methods

2.1. Fluid collection and preparation

2.1.1. Preparation of sheep follicular fluid (FF)

Sheep ovaries of unknown reproductive history (n = 100) were collected from local slaughter house. Ovarian follicles (3–5 mm in

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diameter) were aspirated using disposable syringe (18 gauge). Follicular fluid was left in graduated cylinder to settle. Then FF was transferred to a 10 mL conical tube and allowed to settle for 15 min. The fluid was centrifuged at 3000 rpm for 10 min. Following centrifugation, the obtained supernatant was filtered through a Millipore filter (0.45 μm pore size) and kept in deep freeze at −20 °C till use [16].

2.1.2. Preparation of sheep oviduct fluid (OF)

Oviducts of ewe were collected from El-moneib abattoir at Giza province (n = 200) and were transported within 2 h to the laboratory in 0.9% normal saline supplemented with antibiotics (100 μg/mL streptomycin plus 100 IU/mL penicillin) at 25 °C. Oviducts from ewes in follicular phase of oestrous cycle were used in the present study. The oviducts were dissected free from adjacent connective tissue and washed with normal saline. They were held by artery forceps and their contents were squeezed out by a pair of forceps into a centrifuge tube containing chilled phosphate buffer saline (PBS) with above mentioned antibiotics. The OF was centrifuged at 3000 rpm for 10 min, allowed to sediment to remove debris. The supernatant was filtered, distributed into small eppendorf tubes and stored in deep freeze at −20 °C until use [17].

2.2. Sperm preparation and culture procedure

2.2.1. Collection of semen

Semen samples were collected using artificial vagina from three Barki rams aged 2–3 years, housed indoors at Animal Reproduction Research Institute Farm, Egypt. They were kept under identical conditions of management, feeding and watering throughout the study period. A total of 40 ejaculates were studied during this period (10 weeks). Immediately after collection of semen, the samples were kept in a water bath at 37 °C and transported to the laboratory. Ejaculates contained a volume of 0.5−2 mL, minimum semen concentration of 3×10^9 spermatozoa/mL, progressive motility (>80%); total abnormalities (<10%) were used for the present experiments according to the method described by Chemineau et al. [18]. The samples were pooled to eliminate the individual ram effect.

2.2.2. Sperm capacitation

Split fractions (0.1 mL) of ram semen were transferred into 1 mL of capacitation medium (S-TALP), [19] enriched with various levels of OF or FF or follicular fluids according to experimental design. In experiment 1, the effect of adding 10, 20, 40, 50, 75 μL/mL of OF to capacitation medium on ram sperm functions including progressive motility, hyperactivity and acrosome reaction, was studied. In experiment 2, the effect of different levels FF(10, 20, 40, 50,100, 150, 200 μL/mL) during capacitation on the previous ram sperm functions was assessed. Semen diluted in S-TALP medium without treatment was used as a control. Swim up technique was performed in 15 mL centrifuge tubes, held at a 45° angle and incubated in CO_2 incubator (37 °C). Individual motility percentage (IM %), hyperactivity percentage (HA %) and acrosome reaction (AR %) were recorded at 1, 2, 3 and 4 h post-incubation.

2.2.2.1. Evaluation of sperm motility and hyperactivity. For evaluation of (IM%), 200 μL of the sperm aliquots were placed on clean glass slides, covered by a cover slip and examined under phase contrast microscope (40×) equipped with a heated stage (37 °C). Only progressive forward motility was considered among different treatments and control. At least 100 spermatozoa in five different fields were counted. Percentages of hyperactivated spermatozoa (HA%) were determined by recording the percentage of sperm cells with flagella beating vigorously and circular movement. Hyperactivated motility percentage was considered from the percentage of IM% and expressed by "pluses", where (+) means HA% <20%, + means HA% 20–40%, ++ means HA% 40–60%, +++ means HA% 60–80% and ++++ means HA% >80% as recorded before [20,21].

2.2.2.2. Evaluation of sperm acrosomal status. Percentage of none and complete acrosomal reacted spermatozoa were determined by silver nitrate staining technique according to El-Amrawi et al. [22].The sperm suspensions were spread over microscope glass slides and left at room temperature to dry. The slides were fixed in ethyl alcohol (70%) and followed by fixing spermatozoa in ethyl alcohol (95%) for another 2 min. The samples were then stained with a silver nitrate (1%) solution for 2 h in an incubator at 65 °C, with complete humidity. After the color of the samples turned into gold, the reaction was stopped and the slides were rinsed several times with distilled water and dried at room temperature. The stained preparations were analyzed for acrosome reaction using Olympus BX50 light microscope with a 100-fold magnification under oil immersion lens. To estimate the proportion of acrosome reactions, one hundred of spermatozoa were randomly counted from each selected stained fields. The spermatozoa were classified into three groups; (1) Spermatozoa with an intact plasma and outer acrosomal membrane, (2) Spermatozoa with incomplete acrosome reaction AR showing fenestrations, vesiculation and loosening between plasma membrane and outer acrosomal membrane and (3) Spermatozoa with complete AR showing complete loss of the outer acrosomal membrane leaving cup-shaped appearance. Incomplete plus complete acrosome percentages were considered collectively as total acrosome.

3. Evaluation of the fertilizing capacity of the treated ram spermatozoa

3.1. Collection of ovaries and oocyte recovery

The ovaries of native breed of sheep of unknown reproductive history (1–4 years old) were collected from El-Moneib abattoir. The ovaries (n = 100) were separated shortly after slaughter of animals, dissected away from the surrounding tissues and maintained in a thermo box containing a pre-warmed sterile saline (37 °C) supplemented with antibiotic (100 IU/mL penicillin and 100 μg/mL streptomycin). The average transport time of the ovaries to the laboratory was 2 h. At the laboratory, the ovaries were then washed with warm sterile saline to remove adhering blood. Oocytes were harvested from the ovaries by aspiration technique using 20 G needle fitted to a 5 mL disposable syringe containing a small volume of M-PBS (modified phosphate buffer saline) supplemented with 10% FCS (Fetal calf serum. Sigma, USA). The clear aspirated fluid was poured in Petri dish containing hormone free TCM-199 media examined and by stereomicroscope under low magnification (10×–20×) for the presence of oocytes. Oocytes with a complete corona layer, at least two compact cumulus cell layers and with a homogenous granulated ooplasm (COCs) were used in this study. COCs were washed three times in TCM-199 enriched with 10% FCS plus 1% antibiotic – antimycotic [23].

3.2. In vitro oocyte maturation (IVM) and fertilization (IVF)

Group of 10–15 selected oocytes (COCs) were cultured in minidrops of 50 μL TCM-199 with the above mentioned additives covered with equilibrated sterile Millipore filtered (0.45 μm) lightweight paraffin oil and incubated in CO_2 incubator in 5% CO_2 and 95% relative humidity at 39 °C for 24 h. Then the oocytes were assessed for maturation signs. The oocytes showing expanded cumulus cells, or extruded first polar body in perivitelline space were subjected for IVF [24]. The IVF of the matured oocytes was

carried out as previously described [25]. The cumulus cells were partially removed by gentle pipetting. The matured COCs were washed three times in S–TALP medium. Five to 10 oocytes were then allocated to each minidrop, containing F-TALP medium, in polystyrene culture dish covered with warm sterile paraffin oil and kept in incubator for 1 h until spermatozoa were added. Pretreated sperm with best concentration of OF (50 μL) or FF (40 μL) were added at final concentration of 2×10^6 sperm/mL. Oocytes and spermatozoa were incubated together for 18–24 h at 39 °C under 5% CO_2 in air and at maximum humidity. At the end of the incubation period, presumptive zygotes were examined for the evidence of fertilization. Oocytes with male and female pronuclei ± s econdary polar bodies were categorized to be fertilized. Oocytes with more than one sperm head or male pronuclei were considered to be polyspermic. The rates of fertilization and penetration were recorded.

4. Statistical analysis

Data were expressed as mean ± SEM. Each cconcentration was replicated at least ten times. Data for motility, hyperactivity and acrosome reaction were analyzed by (two way ANOVA) to clarify the effect of various concentrations of OF and FF beside incubation times using *Costat* computer program; version 3.03, Copyright (1986) Cottort Software. Statistical differences among means ($P < .05$) were identified using Duncan's multiple range test (DMRT). On the other hand in vitro penetration and fertilization rates were analyzed by using Chi-square analysis (χ^2).

5. Results

5.1. Effect on sperm motility (IM %)

No significant difference was reported between control and treated OF groups on sperm motility at the beginning of the experiment. The motility was ranged from 79.5% to 83.5%. A significant ($P < .05$) increase in IM% was noticed at concentration of 10 and 50 μL of oviduct fluids as compared to other treatments after 1 h of incubation. A gradual decrease was recorded in the percentage of total motility among different concentrations after 3 to 4 h of incubation (Table 1). Addition of low concentrations of FF (10, 20, 40 and 50 μL/mL) to semen samples resulted in significant ($P < .05$) increase in motility than that treated with higher concentrations (100, 150 and 200 μL/mL). Irrespective to FF levels, the incubation of semen samples at 37 °C for 4 h tended to decline in sperm motility (Table 2).

5.2. Effect on sperm hyperactivity (HA %)

It was clear that addition of 50 μL/mL OF to semen samples resulted in a significant ($P < .05$) increase in HA% (5.60 ± 0.16) compared with those in control group and other concentrations after 2 h sperm incubation. (Table 3). As shown in Table 4, the highest HA % was observed at 50 μL/mL FF after one hour of incubation period. Irrespective to FF concentrations, the HA% significantly increase after 2 and 3hrs of incubation while there was a significantly decline in HA% at 4 h of incubation period.

5.3. Effect on percentages of total acrosome reaction (AR %)

Maximum improvement in AR % was noticed in sperm samples treated with 50 μL/mL OF and maintained up to 3 h of incubation than those observed in other groups (Table 5). Moreover, the total AR% was significantly ($P < .05$) improved as the incubation time increased as illustrated in (Table 5). A similar finding was observed in FF. The highest total AR% was achieved at 40 μL/mL as shown in (Table 6). There was significant ($P < .05$) effect of FF and incubation time on the total AR % of ram spermatozoa.

5.4. Effect on in vitro fertilization

No significant difference could be detected between OF and FF on penetration and fertilization rates of ram spermatozoa. However the rates of the penetration and fertilization of ovine oocytes by sperm previously capacitated with OF or FF were significantly ($P < .05$) higher than those obtained in the control one (Table 7).

Table 1
Effect of different oviduct fluid (OF) concentrations (μL/mL) and incubation time (h) on motility of ram spermatozoa (Means ± SE).

Concentrations of OF	Incubation time (h)				
	0	1	2	3	4
Control	83.0 ± 0.81^{Aa}	80.0 ± 0.00^{Aa}	78.0 ± 0.81^{Aa}	69.0 ± 0.83^{Ab}	32.5 ± 1.69^{Ac}
10	83.5 ± 0.76^{Aa}	79.5 ± 1.16^{Aa}	79.0 ± 1.24^{Aa}	69.5 ± 1.57^{ABb}	52.0 ± 4.54^{ABc}
20	81.5 ± 1.30^{Aa}	76.0 ± 1.45^{ABab}	71.5 ± 0.76^{Bb}	62.0 ± 2.00^{Cc}	36.0 ± 3.23^{CDd}
50	81.0 ± 0.66^{Aa}	80.5 ± 1.38^{Aa}	79.5 ± 1.16^{Aa}	72.0 ± 1.79^{ABb}	57.5 ± 3.18^{Dc}
75	79.5 ± 1.38^{Aa}	73.5 ± 2.24^{Bb}	66.5 ± 1.30^{Cc}	65.5 ± 1.38^{BCc}	44.5 ± 2.03^{BCd}

Means with different alphabetical superscripts in the same columns A, B, C, D and different rows a, b, c are significant at least at $P < .05$.

Table 2
Effect of different follicular fluid (FF) concentrations (μL/mL) and incubation time (h) on motility of ram spermatozoa (Mean ± SE).

Concentrations of FF	Incubation time				
	0	1	2	3	4
Control	79.5 ± 0.50^{Ca}	77.0 ± 0.81^{Aa}	75.0 ± 1.49^{Aa}	64.5 ± 2.62^{Bb}	41.5 ± 3.16^{Bc}
10	84.5 ± 0.50^{Aa}	80.0 ± 1.05^{Aab}	75.0 ± 1.05^{Ab}	78.5 ± 2.47^{Aab}	57.0 ± 4.36^{Ac}
20	84.0 ± 0.66^{Aa}	80.0 ± 1.05^{Aab}	75.0 ± 1.49^{Ab}	73.0 ± 2.49^{ABb}	52.0 ± 4.54^{ABc}
40	84.5 ± 0.50^{Aa}	79.0 ± 1.24^{Ab}	71.5 ± 1.50^{Ac}	75.0 ± 2.68^{ABbc}	45.5 ± 1.38^{ABd}
50	84.0 ± 0.66^{Aa}	80.0 ± 1.05^{Aab}	76.5 ± 1.83^{Abc}	73.5 ± 2.11^{ABc}	52.5 ± 1.53^{ABd}
100	83.0 ± 0.81^{Aa}	76.0 ± 1.24^{Ab}	73.0 ± 1.33^{Ab}	65.0 ± 1.49^{Bc}	40.0 ± 2.58^{Bd}
150	80.0 ± 1.05^{BCa}	79.0 ± 1.24^{Aa}	70.0 ± 1.82^{Ab}	74.5 ± 3.37^{Bab}	44.5 ± 2.40^{Bc}
200	82.0 ± 0.81^{ABa}	75.5 ± 1.16^{Aab}	74.0 ± 1.24^{Aab}	71.0 ± 4.13^{ABb}	39.5 ± 3.37^{Bc}

Means with different alphabetical superscripts in the same columns A, B, C, D and different rows a, b, c are significant at least at $P < .05$.

Table 3
Effect of different oviduct fluid (OF) concentrations (μL/mL) and incubation time (Hours) on hyperactivity of ram spermatozoa (Means ± SE).

Concentrations of OF	Incubation time (h)				
	0	1	2	3	4
Control	0.0 ± 0.00^{Ca}	0.0 ± 0.00^{Da}	0.0 ± 0.00^{Da}	1.0 ± 0.00^{Ca}	1.0 ± 0.00^{Da}
10	0.0 ± 0.00^{Cd}	1.4 ± 0.26^{Cc}	2.6 ± 0.26^{Cb}	3.6 ± 0.26^{ABa}	2.2 ± 0.24^{BCb}
20	0.6 ± 0.26^{BCc}	2.2 ± 0.38^{BCb}	4.0 ± 0.29^{Ba}	3.8 ± 0.24^{Aa}	2.4 ± 0.16^{ABb}
50	2.0 ± 0.20^{Ad}	4.4 ± 0.44^{Ab}	5.6 ± 0.16^{Aa}	4.2 ± 0.24^{Ab}	2.8 ± 0.13^{Ac}
75	0.8 ± 0.24^{Bd}	2.6 ± 0.16^{Bb}	4.0 ± 0.20^{Ba}	3.0 ± 0.20^{Bb}	1.8 ± 0.13^{Cc}

Means with different alphabetical superscripts in the same columns A,B,C,D and different rows a,b,c are significant at least at $P < .05$.

Table 4
Effect of different follicular fluid concentrations (μL/mL) and incubation time (h) on Hyperactivity of ram spermatozoa (Mean ± SE).

Concentrations of FF	Incubation time (h)				
	0	1	2	3	4
Control	0.0 ± 0.00^{Ac}	0.0 ± 0.00^{Cc}	0.0 ± 0.00^{Bc}	1.4 ± 0.26^{Ba}	0.6 ± 0.16^{Bb}
10	0.0 ± 0.00^{Ac}	3.0 ± 0.29^{Bb}	5.6 ± 0.49^{Aa}	5.8 ± 0.61^{Aa}	3.2 ± 0.24^{Ab}
20	0.0 ± 0.00^{Ac}	2.6 ± 0.33^{Bb}	5.8 ± 0.67^{Aa}	5.0 ± 0.59^{Aa}	3.0 ± 0.42^{Ab}
40	0.2 ± 0.13^{Ac}	3.0 ± 0.59^{Bb}	5.2 ± 0.24^{Aa}	5.8 ± 0.61^{Aa}	3.4 ± 0.16^{Ab}
50	0.4 ± 0.26^{Ac}	4.6 ± 0.39^{Aa}	5.4 ± 0.16^{Aa}	4.8 ± 0.57^{Aa}	3.0 ± 0.20^{Ab}
100	0.2 ± 0.13^{Ad}	3.2 ± 0.48^{Bbc}	5.0 ± 0.42^{Aa}	3.8 ± 0.13^{Ab}	2.6 ± 0.16^{Ac}
150	0.4 ± 0.26^{Ac}	3.8 ± 0.43^{ABb}	6.0 ± 0.42^{Aa}	5.0 ± 0.51^{Aa}	3.2 ± 0.13^{Ab}
200	0.8 ± 0.32^{Ac}	2.4 ± 0.33^{Bb}	6.2 ± 0.74^{Aa}	5.8 ± 0.48^{Aa}	2.8 ± 0.24^{Ab}

Means with different alphabetical superscripts in the same columns A, B, C, D and different rows a, b, c are significant at least at $P < .05$.

Table 5
Effect of different oviduct fluid concentrations (μL/mL) and incubation time (h) on total acrosome reaction of ram spermatozoa (Mean ± SE).

Concentrations of OF	Incubation time (h)				
	0	1	2	3	4
Control	0.0 ± 0.0^{Ac}	0.0 ± 0.0^{Cc}	0.0 ± 0.0^{Ec}	8.9 ± 0.67^{Eb}	15.4 ± 2.56^{Da}
10	0.0 ± 0.0^{Ad}	0.0 ± 0.00^{Cd}	8.2 ± 1.0^{Dc}	29.4 ± 3.21^{Db}	41.7 ± 1.93^{Ca}
20	0.0 ± 0.0^{Ad}	7.0 ± 0.86^{Bc}	26.1 ± 3.19^{Cb}	51.1 ± 2.53^{Ca}	53.8 ± 1.62^{Ba}
50	0.0 ± 0.0^{Ad}	0.0 ± 0.00^{Cd}	84.1 ± 2.0^{Ab}	92.5 ± 1.55^{Aa}	71.4 ± 2.63^{Ac}
75	0.0 ± 0.0^{Ae}	11.6 ± 1.07^{Ad}	37.0 ± 2.64^{Bc}	70.7 ± 2.58^{Ba}	54.3 ± 4.48^{Bb}

Means with different alphabetical superscripts in the same columns A, B, C, D and different rows a, b, c are significant at least at $P < .05$.

Table 6
Effect of different follicular fluid concentrations (μL/mL) and incubation time (h) on total acrosome reaction of ram spermatozoa (Mean ± SE) .

Concentrations of FF	Incubation time (h)				
	0	1	2	3	4
Control	0.0 ± 0.00^{Bc}	0.0 ± 0.00^{Ec}	7.0 ± 1.18^{Fb}	16.7 ± 0.95^{Ba}	17.2 ± 1.95^{Da}
10	0.0 ± 0.00^{Bd}	4.1 ± 0.68^{DEd}	24.7 ± 1.93^{Ec}	68.0 ± 4.69^{Aa}	58.3 ± 2.76^{Cb}
20	0.0 ± 0.00^{Bd}	6.4 ± 1.03^{Dd}	36.1 ± 1.66^{Dc}	72.50 ± 4.31^{Aa}	63.1 ± 3.11^{BCb}
40	0.0 ± 0.00^{Bd}	34.6 ± 2.12^{Ac}	62.4 ± 3.02^{Ab}	74.8 ± 2.98^{Aa}	80.0 ± 3.53^{Aa}
50	0.0 ± 0.00^{Bd}	17.6 ± 1.87^{BCc}	30.9 ± 1.84^{DEb}	66.1 ± 2.11^{Aa}	63.1 ± 2.75^{BCa}
100	0.0 ± 0.00^{Bd}	21.6 ± 2.45^{Bc}	46.4 ± 3.01^{BCb}	77.3 ± 4.02^{Aa}	75.3 ± 2.81^{Aa}
150	1.6 ± 0.33^{Bd}	14.0 ± 1.32^{Cc}	39.1 ± 3.67^{CDb}	69.3 ± 2.55^{Aa}	72.6 ± 2.86^{ABa}
200	11.1 ± 1.29^{Ae}	32.2 ± 1.95^{Ad}	50.1 ± 4.03^{Bc}	71.4 ± 2.37^{Aa}	62.2 ± 4.01^{BCb}

Means with different alphabetical superscripts in the same columns A, B, C, D and different rows a, b, c are significant at least at $P < .05$.

Table 7
Effect of best concentration of oviduct fluid (50 μL/mL) and follicular fluid (40 μL/mL) on in vitro fertilization of ovine oocytes.

Capacitating Compounds	Number of matured oocytes	Penetration rate (%)	Fertilization rate (%)
Control	90	$40/90(44.4)^{b}$	$15/40(37.5)^{b}$
Oviduct fluid	100	$74/100(74.0)^{a}$	$55/74(74.3)^{a}$
Follicular fluid	100	$80/100(80.0)^{a}$	$60/80(75.0)^{a}$

Means with different superscripts in the same columns a, b are significant at least at $P < .05$

6. Discussion

In present the study, addition of 50 μL/mL of OF resulted in a marked increase in motility % after 2 h incubation when compared to control one. The maximum rates of HA and AR% were obtained when ram spermatozoa were treated with 50 μL/mL oviductal secretion after further incubation up to 4 h. The present finding showed that, the optimum concentration of OF to be used in IVF at level 50 μL/mL. In this respect, Parrish et al. [26] successfully induced capacitation and AR in bovine spermatozoa using oviduct

fluid at time of estrus within 4 h of incubation in vitro. Furthermore, McNutt and Killian [27] observed that, the pooling samples of oviduct fluid from mature cow (non luteal and luteal) maintained 80% of motility of sperm for up to 6 h. They added that, the percentage of capacitated sperm treated by OF obtained from non-luteal ovary were higher than those capacitated in luteal one (60 and 80%, respectively). Several researches indicated that the oviduct secretions contain many proteins which enhance viability of sperm, increase sperm motility, keep membrane intact and promoted sperm capacitation [26,28–31]. Recent evidence indicates that the oviduct secretions contain many components such as oviduct-specific glycoproteins, glycosaminoglycans, carbohydrates; norepinepherine, catecholamines, heat-shock protein, and osteopontin that have the affinity to modulate sperm function [8,9]. With respect to incubation times, a significant ($P < .05$) increase in total AR% was also noticed herein after incubation for 3 h (92.5%) without needing any substances. However, the rates of AR of ram sperm obtained in OF for 4 h incubation were 22–24% [32]. The difference in methodology and samples used has probably resulted in the disparate results obtained. However, Lapointe et al. [29] reported that, OF was not enough to induce acrosome reaction of bovine sperm unless sperm treated with inducing substances like lysophosphatidylcholine, solubilized zonae pellucidae, or FF. These results are not supported by the present study where, this difference may be due to species difference. The present study revealed that a very low levels of FF significantly increased motility of ram spermatozoa with highest HA% was observed at 50 μL/mL. On other hand, supplementation of two concentrations of bovine follicular fluid (25% or 50%) (FF) to Krebs Ringer phosphate buffer enriched with 5% fructose resulted in marked increase of bull sperm motility after 9 h incubation kept at 37 °C [13]. Although the incubation of ram spermatozoa with different concentrations of FF for long time (4 h) decreased the sperm motility in the present finding, it increased hyperactivity at a concentration of 40 to 50 μL/mL. This may be attributed to change of motility from progressive forward motility to circular one with flagellar beating vigor as indication of hyperactivity [21]. In the present finding, the total AR% was significantly ($P < .05$) improved as 40 μL/mL (80.00 ± 3.53). Follicular fluid has been known for many years to be a rich source of gylcosaminoglycans. It has been proposed that gylcosaminoglycans in FF, or associated with cumulus cells, may reach sperm in the oviduct and induce the AR [29]. In addition, G-75 fraction obtained from human FF stimulates hydrolysis of membrane phospholipids in the presence of calcium leading to acrosome reaction [33]. Lipid transfer activity in FF and OF may lead to loss of cholesterol from sperm membrane which results in capacitation [34]. The fertilizing capacity of spermatozoa is the target of capacitation process, thus the ability of ram spermatozoa, treated with the best concentration of FF or OF to penetrate and fertilize matured ovine oocytes in vitro. It was clear that the rates of penetration and fertilization of oocytes inseminated with spermatozoa treated with FF or OF were significantly higher compared to than those obtained in the control one (untreated). Similar finding was observed by [35] who concluded that goat follicular fluid (gFF) at 10% concentration has a positive effect on in vitro fertilization and embryo development of Black Bengal goat oocytes. On other hand, El-Shahat [15] observed that incubation of buffalo-bull semen for 1 h in 25% buffalo follicular fluid before addition of matured oocytes elevated relatively the fertilization rate (34.7%).

7. Conclusion

In conclusion, treatment of ram spermatozoa with 50 μL/mL of OF or 40 μL/mL of FF for 3 to 4 h incubation respectively was considered the best concentration of oviduct or follicular fluid to be used for IVF.

Conflict of interest

No conflict of interest.

References

[1] Florman HM, Babcock DF. Progress towards understanding the molecular basis of capacitation. In: Wassarman PM, editor. Elements of mammalian fertilization. Basic concepts, vol. 1. Boca Raton, FL: CRC Press; 1991. p. 105–32.
[2] Cohen-Dayag A, Eisenbach M. Potential assays for sperm capacitation in mammals. Am J Physiol Cell Physiol 1994;267:1167–76.
[3] Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD, editors. The physiology of reproduction. New York: Raven Press; 1994. p. 189–317.
[4] Visconti PE, Galantino-Homer HL, Moore GD, Bailey JL, Ning X, Fornes M, et al. The molecular basis of sperm capacitation. J Androl 1998;19:242–8.
[5] Oliphant G, Ross PR. Demonstration of reproduction and isolation of three sulfated glycoproteins from the rabbit oviduct. Biol Reprod 1982;26:537–44.
[6] Sutton R, Nancarrow CD, Wallace ALC, Rigby NW. Identification of an oestrus associated glycoprotein in oviductal fluid of the sheep. J Reprod Fertil 1984;72:415–22.
[7] Rodriguez-Martinez H. Role of the oviduct in sperm capacitation. Theriologenology 2007;68:138–46.
[8] Killian G. Evidence for the role of oviduct secretions in sperm function, fertilization and embryo development. Anim Reprod Sci 2004;82–83:141–53.
[9] Killian G. Physiology and endocrinology symposium: evidence that oviduct secretions influence sperm function: a retrospective view for livestock. J Anim Sci 2011;89(5):1315–22.
[10] Fortune JE. Ovarian follicular growth and development in mammals. Biol Reprod 1994;50:225–32.
[11] Angelucc S, Ciavardelli D, Di Giuseppe F, Eleuterio E, Sulpizio M, Tiboni GM, et al. (2006): Protone analysis of human follicular fluid. Biochim Biophys Acta 2006;1764:1775–85.
[12] Foley CW, Williams WL. Effect of bicarbonate and oviduct fluid on respiration of spermatozoa. Proc Soc Exp Biol Med 1967;126:634–7.
[13] Breuer DJ, Wells ME. Effects of in vitro incubation of bovine spermatozoa in bovine follicular fluid. J Anim Sci 1977;44:262–5.
[14] Hammam AM. Effect of addition of sera, bovine oviduct epithelial and granulose cells to culture medium on maturation, fertilization and development of cattle oocytes in vitro. Egypt J Appl Sci 1997;12(5):35–50.
[15] El-Shahat KH. Effect of addition of follicular fluid, oviductal or granulose cell co-culture on cleavage and embryonic development of buffalo oocytes. Vet Med J Giza 2011;59(4):33–8.
[16] Tajik P, Beheshti-Govij R, Soleimani-Rad J, Ghasemzadeh-Nava H, Mohamadi-Roshandeh A. Effects of different concentrations of bovine follicular fluid and estrous cow serum on development of murine 2-cell embryos. Pak J Biol Sci 2008;11:628–32.
[17] Imam S, Ansari MR, Ahmed N, Kumaresan A. Effect of oviductal proteins on buffalo sperm characteristics during cryopreservation. Theriologenology 2008;69:925–31.
[18] Chemineau P, Cognine Y, Guerin Y, Orgeure P, Valtet JC. Training Manual on Artificial Insemination in sheep and goats. FAO Animal Production and Health. 1991; pp no. 83. FAO, Rome, Italy. 222p.
[19] Younis AI, Zuelke KA, Harper KM, Oliveira A, Brackett BG. In vitro fertilization of goat oocytes. Biol Reprod 1991;44:1177–82.
[20] Kawakami E, Arai T, Nakamura U. Effects of medium containing heparin and theophylline on capacitation and metabolic enzyme activities of ejaculated spermatozoa from dogs with asthenozospermia. Anim Reprod Sci 1999;54:251–9.
[21] Darwish GM. Evaluation of fertilizing efficiency of buffalo spermatozoa in vitro subsequent using some capacitating agents. J Egypt Vet Med Assoc 2004;64 (4):323–36.
[22] El-Amrawi GA, Nemetallah BR. Study on capacitation of buffalo spermatozoa. Egyptian Society Animal Reproduction Fertility 3rd Annual Cong. Cairo, 1992; pp. 214–222.
[23] Datta TK, Goswami SL, Das SK. Comparative efficiency of three oocyte recovery methods from sheep ovaries. Indian J Anim Sci 1993;63:1178–9.
[24] Abeydeera LR, Wei-Hua W, Prather RS, Day BN. Maturation in vitro of pig oocytes in protein-free culture media: fertilization and subsequent embryo development in vitro. Biol Reprod 1988;58:1316–20.
[25] Dell' Aquila ME, Cho YS, Minoia P, Traina V, Fuscos S, Lacalandra GM, Maritato F. Intracytoplasmic sperm injection (ICSP) versus conventional IVF on abattoir-derived and in vitro-matured equine oocytes. Theriologenology 1997;47:1139–56.
[26] Parrish JJ, Susko-Parrish JL, Handrow RR, Sims MM, First NL. Capacitation of bovine spermatozoa by oviductal fluid. Biol Reprod 1989;40:1020–5.
[27] McNutt TL, Killian GJ. Influence of bovine follicular and oviductal fluids on sperm capicitation in-vitro. J Androl 1991;12:244–52.
[28] Wegner CC, Killian GJ. Origin of oestrus associated glycoproteins in bovine oviductal fluid. J Reprod Fertil 1992;95:841–54.

[29] Lapointe S, Buhr MM, Ahmad I, Lambert RD, Sirard MA. Modulation of post thaw motility, survival, calcium uptake and fertility in bovine sperm by female genital products. J Dairy Sci 1996;79:2155–62.
[30] Kumaresan A, Ansari MR, Garg A. Modulation of post thaw sperm functions with oviductal proteins in buffaloes. Anim Reprod Sci 2005;90:73–84.
[31] Kumaresan A, Ansari MR, Garg A. Effect of buffalo oviductal and follicular fluid proteins on in vitro sperm capacitation. Indian J Anim Sci 2006;76:204–7.
[32] Chavarría ME, Reyes A. Secretions of ovine uterus and oviduct induce in vitro capacitation of ram spermatozoa. Arch Androl 1996;36(1):17–23.
[33] Thomas P, Meizel S. An influx of extracellular calcium is required for initiation of the human sperm acrosome reaction induced by human follicular fluid. Gamete Res 1988;20:397–411.
[34] Ravink SE, Killian GJ, Muller CH. Bovine reproduction fluids have lipid transfer protein and stimulate human sperm capacitation. Biol Repod 1990;1(Suppl. 1):86.
[35] Masudul Hoque SA, Yahia Khandoker MAM, Kabiraj SK, Asad LY, Fakruzzaman M, Tareq KMA. Effect of goat follicular fluid on *in vitro* production of embryos in black bengal goats. Iran J Appl Anim Sci 2012;2(3):287–94.