ORIGINAL RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 9 | September 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

In Vitro Fertilization of Buffalo Follicular Oocytes by Intravaginal Culture in Bovine Vagina

KEYWORDS: Buffalo, oocytes, in vitro fertilization, intravaginal culture

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ABSTRACT *In 2 experiments we compared the in vitro maturation and fertilization of buffalo follicular oocytes in laboratory culture and intravaginal culture. The bovine vagina was used for vaginal culture of oocytes that were loaded in empty semen straws incorporated in empty progesterone vaginal implants (CIDR, Inter AG) and placed intravaginally. The in vitro maturation (Expt 1) and subsequent fertilization rates (Expt 2) were significantly higher in laboratory culture (74.54% and 31.34%) compared to those obtained in intravaginal culture (38.18% and 11.47%) however oocytes could successfully be matured and fertilized in vitro in the vaginal culture. It was concluded that intravaginal culture can be successfully used for in vitro maturation and fertilization of buffalo oocytes.*

Introduction

Intravaginal oocyte culture offers the advantage of in vitro culture of oocytes without a laboratory set up and has been used in human subjects[1-3]. The technique utilized in human placed 1 to 5 retrieved oocytes in a tube filled with 3 mL of culture medium along with 10-20 thousand sperms/mL prepared previously. The tube was sealed and placed in maternal vagina and held by a diaphragm for 44 to 50 h[1,2]. For culture of bovine oocytes Moyo and Dobson[4] placed them on capsules which were kept intra-vaginally in the bovine vagina. Buffalo oocytes were placed in the bovine vagina for intra-vaginal culture by filling in empty semen straws that were attached to empty used CIDR devices[5] however; the oocytes were only matured in vitro in this report. In this report we present the results of in vitro fertilization of buffalo oocytes by intravaginal culture.

Materials and Methods

Buffalo ovaries obtained from a abattoir were transported to the laboratory in warm normal saline solution supplemented with antibiotics in an isothermic container. Surface follicles were aspirated from the ovaries using an 18 G needle attached to a syringe. Oocytes with a multilayered cumulus cells and an even cytoplasm were located under a stereomicroscope and washed twice in TCM-199 (Sigma Chemicals USA) supplemented with Hepes and antibiotics. Oocytes were placed in the maturation medium comprising of TCM199 supplemented with 0.25mM pyruvate, 25mM Hepes, 5µg/mL follicle stimulating hormone, 5µg/mL luteinizing hormone, 1µg/mL estradiol and antibiotics. For laboratory culture, the oocytes were cultured in 100µL droplets in Petri dishes (Becton Dikinson) under oil in a humidified atmosphere in a CO_2 incubator. For intravaginal culture the oocytes were cultured as per methods described previously[5]. Briefly oocytes were loaded in 0.5 cm^3 pre-sterilized empty semen straws and the sealed semen straw was placed in an autoclaved used CIDR implant (Inter Ag, Holland). The CIDR along with the semen straw was placed (using the applicator) in the vagina of a mid luteal phase cow and kept for the culture period i.e 24 h.

In Experiment 1 after 24 h of culture both groups of oocytes were taken out and all the oocytes were evaluated for nuclear maturation by fixation with acetic methanol on a slide and staining with aceto-orcein as described previously[6]. In Experiment 2, after in vitro maturation the oocytes were processed for in vitro fertilization. The oocytes were fertilized with prepared sperm as per methods described previously[7]. Briefly 4 mL of 90% isotonic Percoll was layered in a 15 mL centrifuge tube beneath 4 mL of 45% isotonic Percoll. The sperms were initially washed in TALP-BSA by centrifugation at 250 g for 4 min. The sperm pellet was resuspended in 1 mL of the medium. The washed sperm pellet was layered on the top of Percoll gradient and centrifuged at 300 g for 35 min. The resultant pellet was removed from the bottom and washed twice in TALP-BSA by centrifugation. The sperm pellet was resuspended in TALP to give a final concentration f 1-2 million sperms. This was incubated for 2-3 h in a CO2 incubator. The matured oocytes were transferred to another dish containing Fert-TALP (TALP supplemented with 30 µg/mL penicillamine, 15 µmol/mL hypotaurine, 10 µg/mL heparin and 1 µmol/mL adrenaline) under paraffin oil. They were inseminated with sperms in a volume so as to give a final concentration of 1-2 million sperms. Oocytes with sperm were co-incubated in the vagina or the CO_2 incubator as done previously for in vitro maturation. Following 20-24 h of sperm oocyte coincubation the oocytes from each group were fixed for assessment of fertilization as described previously[6].

Results and Discussion

Significantly higher proportions of oocytes were matured and fertilized in vitro in laboratory culture compared to the intravaginal culture however oocytes kept in intravaginal culture also matured and were fertilized in vitro subsequently. Similar to our previous findings the empty progesterone vaginal implants carried the straws with oocytes in the bovine vagina that served as a biological incubator with probably conditions conducive to oocyte growth and fertilization. In terms of in vitro maturation rates of oocytes the present results are similar to our previous findings in buffalo[5]. In terms of fertilization rates observed in the pre-

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sent study similar fertilization rates of 11-24% have been recorded in many previous studies on buffalo oocyte fertilization[8-10]. Although a few studies on buffalo recorded higher in vitro maturation and fertilization rates compared to the present study[6,11] but these studies used different media and culture combinations. Although in comparison to laboratory culture in vitro maturation and fertilization rates are lower in the intravaginal culture the ease of the use of the technique suggests the usefulness at places where a incubator is not available. It was concluded that intravaginal culture can be successfully used for in vitro maturation and fertilization of buffalo oocytes.

Table 1. Nuclear maturation and in vitro fertilization rates of buffalo oocytes in laboratory culture and intravaginal culture.

Culture	Experiment 1				Experiment 2			
	No of replicates	No of oocytes	No reaching M-II	Proportion matured	No of replicates	No of oocytes	No of oocytes fertilized	Proportion fertilized
Laboratory	6	55	41	74.54%[a]	8	67	21	31.34%[a]
Intravaginal	10	110	42	38.18%[b]	11	122	14	11.47%[b]

Proportions with different superscripted letter differ significantly (P<0.01)

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