Research Article
CODEN (USA): IJPLCP

[Alwan & Saleem, 4(2): Feb., 2013]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Effect of heparin on the capacitation of frozen – Thawed bull sperm used in the *In-vitro* fertilization of oocytes matured *In-vitro*

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Abstract

Ovarian Oocytes were matured and fertilized *in-vitro* with frozen-thawed bull sperm capacitated *in-vitro* .Frozen – thawed sperm were swim up separated in a modified Ca^{++} - free Tyrode's medium and washed twice for 10 min. on each occasion. The washed sperm were then diluted (1:1) with same medium containing heparin so as to give final concentration of 0, 10, 50 and 100 ug/ml. Incubation of heparin treated sperm was carried out at 39C° in 5% Co_2 for a periods of either 15 min. or 30 min. The inseminated Oocytes were cultured at 39C° 5% Co_2 in air for 20-48h and were then fixed, stained and examined for evidence of maturation and fertilization. The results show that a high percentage (96%) of Oocytes to reach metaphase II within 28 hours. It was also observed that the use of heparin was highly effective in enabling sperm to penetrate Oocytes. The proportion of Oocytes fertilized was increased significantly as the concentration of heparin in the capacitation medium increased significantly as the concentration of heparin in the capacitation medium increased (4.8%, 50.9%, 69.3%, and 83.6% for 0,10,50 and 100 µg/ml, respectively; $P<0.01$). The percentage of Oocytes cultured with cow serum developed to 2-4-cells in a significantly greater than those cultured with BSA (63.3% VS 32.1%; $P<0.01$).

Key-Words: Heparin, Thawes bull Sperm, *In-Vitro*

Introduction

The development of an efficient laboratory procedure which would enable cattle ovarian Oocytes to be matured *in-vitro*, fertilized and cultured *in-vitro* to the blastocyst stage of development could have important practical and scientific implications. The commercial exploitation of certain embryo transfer techniques applicable in cattle might be facilitated by the development of such a procedure and there would be many advantages to having a cheap source of embryos available for research purposes and embryo transfer.

Development to term has been achieved after transfer of sheep, goat and cattle oocytes matured and fertilized *in-vitro* to recipient females [1,2,3].

The objective of this study was to examine the effect of heparin on the capacitation of bull sperm used in the *in-vitro* fertilization of bovine oocytes.

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Material and Methods

To obtain Oocytes, ovaries from varied breeds of cows shortly after slaughter were collected from the local slaughter house. The Ovaries are brought to the laboratory in phosphate buffered saline (PBS) held at 30C° within 2 hours of the cows slaughter. Intact follicles (2-6 mm.diameter) are dissected out of the basis of their opacity , vascularization and on the integrity and uniformity of the membrana granulosa. Primary Oocytes are recovered on the rupture of the follicle, care being taken to preserve the integrity of the Oocytes cumulus complex. primary oocytes was matured in medium 199 supplanted with 15% Heat Treated Estrous Cow Serum (HTECS) or Bovine Serum Albumin (BSA), granulose cells(1-5 million/ml) , 15ng FSH, 1 µg LH and 1µg E_2/ml for 24 hours at 39 C° , and using 5% CO_2 in air .

Frozen – thawed bull sperm were swim – up separated in a modified Ca^{++} - free Tyrodes medium (PH 7.4) and washed twice according to methods were previously described (3) . The washed sperm were then diluted (1:1) with the same medium containing heparin so as so give final concentration of 0, 10, 50 and 100 µg/ml . Incubation of heparin treated sperm

was carried out at 39C° in an atmosphere of 5% Co_2 in air for periods of 15 to 30 min.

Fertilization *in-vitro* has been achieved by adding doses of 1-1.5 million sperm /ml of capacitated sperm to groups of matured Oocytes in micro droplets of fertilization medium (modified Tyrode's). The inseminated Oocytes were cultured at 39C° in 5% Co_2 in air for 20h. or cultured *in- vitro* for up to 48h and examined for evidence of fertilization.

Results and Discussion

Developmental Capacity, it is necessary to select Oocytes complement for supporting meiotic maturation and embryonic development, and to use culture conditions that ensure full Oocyte maturation. Data are in table 1 for 800 Oocytes which were cultured in medium 199 supplemented with 15% HTECS and additional granulose cells. In this particular study, culture methods employed were effective in permitting a high percentage (96%) of the Oocytes to reach metaphase II within 28h., comparable to that reported by (4) . As culture period increased from 24h. there is a gradual increase in the proportion of oocytes in second metaphase stage . However, the result presented here is in agreement with report of (5). Generally , maturation of cattle Oocytes tended to increase with time and requires about 30 hours for maturation *in-vitro*(6).

The use of the heparin was highly effective in capacitating sperm as shown by the percentage of oocytes penetrated in table 2. Only frozen- thawed semen in which sperm showed evidence of good motility after thawing was employed. Evidence suggests that most oocytes were penetrated by just one sperm and the incidence of parthenogenetic activation of eggs has been low.

The results show that the use of heparin was highly effective in enabling sperm to penetrate Oocytes (heparin treatment, 69.7%, control, 2.3%; $p<0.01$). Similar observations were reported by (7). The proportion of Oocytes fertilized was increased significantly ($P<0.01$) as the concentration of heparin in the capacitation increase. Results achieved after 15 min. incubation of sperm in the capacitation medium were similar to those after 30 min. incubation. These data were comparable to those reported in the cow for *in-vitro* maturation and fertilized *in-vitro* (7).

A study dealt with in table 3,a comparison was made between heat treated estrous and cow serum (obtained from cattle in natural oestrus) and bovine serum albumin in the medium 199 medium employed in maturing the Oocytes . Results in this study show that the cow serum proved to be superior in terms of the percentage of eggs when had cleaved to the 2-4-cells stage when examined at 48 hours. Normal development of 2 - and – 4 cell stage embryos after *in- vitro* insemination of bovine sperm has been documented with reference to electron microscopy (9).

Conclusion

In conclusions, result indicate that the use of heparin effective in enabling sperm to penetrate Oocytes and that bovine Oocytes derived by in-vitro maturation and IVF procedures are capable to cleavage to 2-4-cells stage. It remains for further studies to establish whether the IVF eggs produced with the present system are capable of normal embryonic and fetal development .

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Table 1: Development of Primary Bovine Oocytes in Culture medium

Period of Culture h.	No. of Oocytes	% of Oocytes in			
		GV	GVBD	MI	MII
0	100	96	4		
4	100	10	90		
8	100		100		
12	100		53	47	
16	100		3	97	
20	100		1	90	
24	100			6	94
28	100			4	96

GV: germinal vesicle; GVBD: GV breakdown; MI: metaphase of the 1st meiotic division; MII: metaphase of the 2nd meiotic division

Table 2: Effect of heparin treatment on percentage of Oocytes penetrated by bull sperm

Heparin Dose µ	No. of secondary Oocytes penetrated in relation to sperm pre-incubation period			
	15min.		30min.	
	No. of Oocytes	penetrd. (%)	No. of Oocytes	penetrd (%)
0	22		21	1(4.8)
10	23	14(60.9)	22	9(40.9)
50	25	19(76.0)	24	15(62.5)
100	31	27(87.1)	30	25(80.0)

Table 3: Effect of serum source on cleavage of Oocytes after IVF

Source of Serum	Oocytes Inseminated	Oocytes Examined	Oocytes Fertilized (%)	Eggs cleaving To the 2-4 cell Stage (%)
BSA	110	102	81(79.4)	26(32.1)
HTECS	115	112	98(87.5)	62(63.3)