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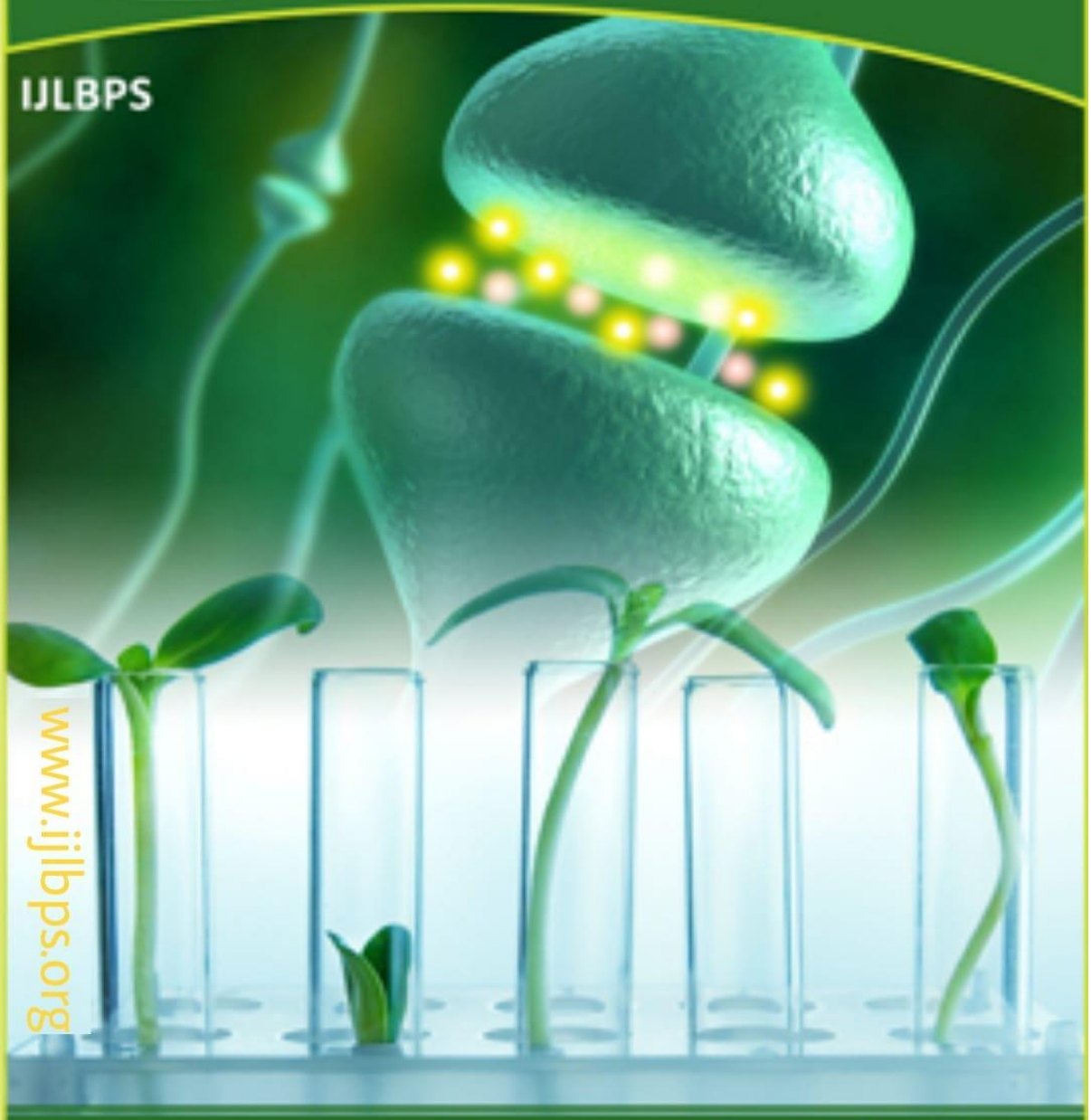

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In vitro fertilization with in vitro developed oocytes: the impact of heparin on the viability of frozen-thawed bull sperm

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Abstract

In-vitro-matured oocytes were fertilized with bull sperm that had been frozen and thawed in-vitro.Swim up separation was performed on thawed, frozen sperm in a Ca++-free variant of Tyrode's medium for a total of 20 minutes. The cleaned sperm were then diluted (1:1) with identical medium containing heparin so as to yield final concentration of 0, 10, 50 and 100 ug/ml. For 15 or 30 minutes, we incubated heparin-treated sperm at 39 degrees Celsius in 5% carbon dioxide.and checked for signs of development and fertilization by fixing, staining, and examining them. The data demonstrate that almost all Oocytes (96%) enter metaphase II by the end of the 28th hour. Heparin was also shown to be quite useful in facilitating sperm penetration of Oocytes. The percentage of fertilized Oocytes rose dramatically with increasing heparin content in the capacitation medium (4.8% for 0 g/ml, 50.9% for 10 g/ml, 69.3% for 50 g/ml, and 83.6% for 100 g/ml; P0.01). Cow serum resulted in a considerably higher rate of oocyte development to 2-4 cells (63.3% vs. 32.1%; P0.01) than BSA.

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

INTRODUCTION :

The successful in vitro maturation, in vitro fertilization, and in vitro culture to the blastocyst stage of development of ovarian Oocytes from cattle might have significant practical and scientific significance. There would be numerous benefits to having a cheap supply of embryos accessible for study and embryo transfer, and the commercial exploitation of some embryo transfer procedures suitable in cattle may be aided by the establishment of such a process.Oocytes developed and fertilized in vitro have been transferred to recipient females from sheep, goats, and cattle, resulting in live births (1, 2, 3).The purpose of this investigation was to determine whether heparin would inhibit the ability of bull sperm to fertilize bovine oocytes in vitro.

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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% HeatTreated 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

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, 50and 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 maturationand embryonic development, and to use culture conditions that ensure full Oocyte maturation. Data are in table 1 for 800 Oocytes which were cultured inmedium 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 isin 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 developmentof 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 heparineffective in enabling sperm to penetrate Oocytes andthat 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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