Date: / /
Page No.:

Gamete & Cryopreservation

→ Gametes are haploid (n) in Nature
→ Male and Female gametes unite to form a Zygote (2n)
→ Gametes contains a single set of chromosomes and hence are haploid in nature.
→ When gametes and embryos are handled in vitro they are to be stored in in liquid nitrogen at -196°C, indefinitely for infinite period

→ Gamete cryopreservation is of for
a) male gamete cryopreservation or Spermatozoa cryopreservation and
b) female gamete cryopreservation or Oocyte cryopreservation

Spermatozoa Cryopreservation

→ Cryopreservation of human semen is well-established laboratory procedure.
→ It helps in maintaining the fertilizing potential of spermatozoa during storage in liquid nitrogen.
→ In comparison with other cell, spermatozoa are not so sensitive.

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Date: / /
Page No.:

to cryopreservation damage.
This is because of the high fluidity of the membrane and the low water content (about 50%).

→ The effect

→ The freezing of sperm is relatively simple and rapid. It does not involve seeding or the use of slow, controlled rates of cooling.

→ First of all we collect the samples.

→ After collection of samples, we perform the process of liquefaction of samples.

→ After that cryoprotectant is added at ambient temperature, usually in a 1:1 ratio.

→ Then give a final glycerol concentration of 50 : 5 to 10%.

→ Cryoprotectant solutions may contain extenders such as egg yolk and pH buffers such as TES and

Date: / /
page No.:

Vials and are available commercially

→ Diluted semen samples are aliquoted (taken in small amount) about 1 ml and transferred to straws or vials for freezing. This freezing is performed in liquid nitrogen at -196°C.

→ Many sperm banks do not use programmable freezers but simply expose samples diluted in cryoprotectant, with or without precooling to liquid nitrogen vapour at -130°C, followed by a plunge into liquid nitrogen at -196°C temperature.

→ A labling and recording system must be in place to prevent sample loss or misidentification misidentification

* Thawing and Cryosurvival

→ For thawing, solutions are prepared and the desired vial or straw is identified and removed from liquid nitrogen storage.

→ With vials, the cap should be unsealed and replaced to

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Date: / /
Page No.:

to prevent trapped nitrogen from expanding during warming, which can result in vial rupture.

→ The sample is warmed by vigorous agitation in a 37°C water bath until the ice disappears.

→ The contents are decanted or removed with a pipet, and the gamete is recovered and transferred to different buffered saline solution and finally to fresh growth medium at 37°C temperature.

Oocyte cryopreservation

→ The successful cryostorage of oocytes could circumvent many of the ethical objections to storing embryos and could provide alternatives to patients whose reproductive capacity is threatened by health concerns.

→ Unfortunately, significant deleterious effects of cryopreservation

Date: / /
Page No.:

oocyte survival and function have been noted, including disruption of the microtubular spindle, leading to aneuploidy after fertilization.

→ Efforts to overcome these limitations by freezing immature oocytes have met with limited success.
Thus, routine cryopreservation has focused on embryos.

Applications of Gamete Cryopreservation

(i) Sperm Gametic cryopreservation is useful to solve fertility problems

NOTE :- Sperm was the first type of reproductive cell successfully frozen. It still remains the easiest cell to freeze because of containing low amounts of cytoplasm and consequently low quantity of water.

(ii) In human & veterinary medicine, preservation of gamete is closely connected with the development of artificial insemination. Artificial insemination is useful in breeding programs.

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Date: / /
Page No.:

(iii) Nowadays tissues, cultured cell lines, DNA, and serum samples could be frozen and stored in cryogenic banks.

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