



International Symposium on
Fertility Preservation
From Basic Research to Clinical Applications

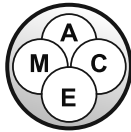


Kasturba Medical College

Manipal - 576104, India

28th February and 1st March 2015

ABSTRACTS



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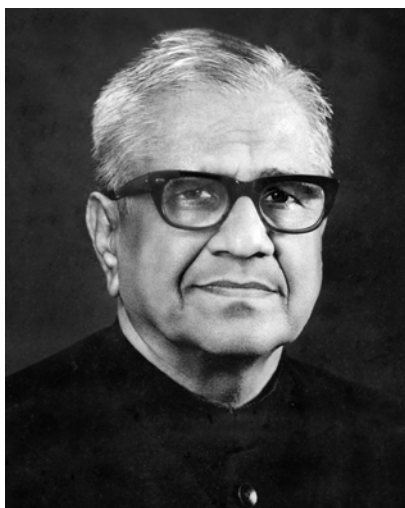


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International Symposium on Fertility Preservation: From Basic Research to Clinical Applications

28th February & 1st March 2015, Manipal

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International Symposium on Fertility Preservation:

From Basic Research to Clinical Applications



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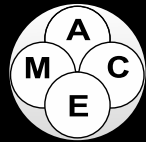
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Manipal Association of Clinical Embryologists (MACE)



Manipal Association of Clinical Embryologists (MACE), a non-profit association based at Manipal, Karnataka State, India is focused forum of Embryologists and researchers working in the area of human reproduction. Conceived on 19th of December 2010 by a group of scientists, who have witnessed the progress of assisted reproduction to every corner of the globe.

The association is committed to support academic activities for the professionals who are engaged in the clinical practice and research in human embryology.

MACE is dedicated to the advancement of the knowledge, and practice of embryology and reproductive medicine by various ways. The Society organizes scientific conferences, symposium, workshops in specific areas, conducts regular seminars from eminent speakers and subsequent dissemination of knowledge to the professionals.

The association also provides various benefits including reduced price registration and accommodation for academic events, travel awards and partial research support to its members.

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Session	Time	Topic	Chair
I	9.00-9.30	Impact of cancer therapy on normal tissues: Clinician's perspective - Krishna Prasad	Vidyasagar/ S Hebbar
I	9.30-10.00	Late effects of treatment in survivors of childhood cancer - Rejiv Rajendranath	
I	10.00-10.30	The effects of cancer treatments on female fertility - Richard Anderson	
	10.40-11.10	Inauguration of the symposium	
I	11.10-11.30	Refreshments and Poster view	
II	11.30-12.00	Medical interventions in protecting reproductive functions during cancer therapy - Rejiv Rajendranath	S Schlatt/ P Kini
II	12.00-12.30	Natural compounds: is it a safe approach to protect gonads from cytotoxic agents?- Guruprasad Kalthur	
II	12.30-1.00	Prepubertal hematological malignancies: practical approaches in fertility preservation - Harsha Prasada	
	2.00	Lunch	
III	2.00-2.30	First experience with ANDROPROTECT - A clinical program to preserve fertility in boys - Stefan Schlatt	R Anderson/ N Pandiyan
III	2.30-3.00	Ethical and social issues in fertility preservation - N Pandiyan	
III	3.00-3.30	Fertility preservation in gynecological practice: Patient's perspective - Kamini Rao	
	3.30-4.00	Refreshments	
IV	4.00-4.15	Informing patients about fertility issues before cancer therapy - Karthik Udupa	R Talevi/ Vijay Kumar
IV	4.15-4.30	Role of oocyte vitrification for fertility preservation programs - Javier H Zapata	
IV	4.30-4.45	Ovarian preservation in surgical management of gynecological cancer - Satadru Ray	
IV	4.45-5.00	Laparoscopic cystectomy in benign ovarian condition and ovarian preservation - Rajesh Bhakta	
	5.00 pm	Departure to Beach Side	

Session	Time	Topic	Chair
V	9.00-9.30	Current methods of fertility preservation - Mats Brannstrom	Kamini Rao/ Pratap Kumar
V	9.30-10.00	Patient selection for fertility preservation - Richard Anderson	
V	10.00-10.30	Human ovarian tissue vitrification in Oncofertility practice - Riccardo Talevi	
V	10.30-11.00	Ovarian cryopreservation in Sweden: clinical results and research efforts - Mats Brannstrom	
	11.00-11.30	Tea and Poster view	
VI	11.30-12.00	Strength and weakness of a testicular stem cell system in man during normal and disturbed gonadal function - Stefan Schlatt	R Anderson/ Satish Adiga
VI	12.00-12.30	Uterus transplantation to restore fertility after cervical cancer surgery - Mats Brannstrom	
VI	12.30-1.00	Embryologist's perspective in handling gametes of cancer survivors in ART - Varsha Roy	
	1.30	Lunch	
VII	1.30-2.15	Panel discussion: Fertility preservation - Practical problems in establishing multidisciplinary network Panelists: R Anderson/I Rubio/ R Rajendranath/ Sujatha R/Harsha Prasada	Moderator: Kamini Rao
VIII	2.15-2.30	Preliminary studies at fertility preservation – An Indian experience - Jyothi Menon	M Brannstrom/ N Malhotra
VIII	2.30-3.00	Restoring fertility in cancer survivors: Ovarian tissue transplantation or ART? - Kamini Rao	
VIII	3.00-3.30	Ovarian stimulation in cancer patients: Approach and challenges - Pratap Kumar	
IV	3.30-3.45	Tea	
IV	3.45-4.15	Semen cryopreservation in fertility preservation program - Sujatha R	Irene Rubio/ Swaminathan
IV	4.15-4.45	Avoiding the transmission of malignant cells: single culture of primary follicles versus culture of ovarian slices - Riccardo Talevi	



INVITED TALKS



Impact of Cancer Therapy on Normal Tissues: Clinician's Perspective

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Cancer is treated with surgery, radiotherapy, chemotherapy and targeted therapies.

The Side Effects of Chemotherapy on the Body:

Cancer cells divide more quickly than healthy cells, and chemotherapy drugs effectively target those cells. Unfortunately, fast-growing cells that are healthy can be damaged too. Some patients experience few side effects while others feel quite ill. Although most side are short term or acute in nature, some may continue well after chemotherapy has ended, and some may be permanent.

Chemotherapy drugs are most likely to affect cells in the digestive tract, hair follicles, bone marrow, mouth, and reproductive system which are fast dividing, however, cells in any part of the body may be damaged.

Circulatory and Immune Systems: Drugs can harm cells in the bone marrow, which can result in several problems. Anemia occurs when body doesn't produce enough red blood cells causing fatigued. Chemotherapy can lower white blood cell count (neutropenia), which raises the risk of infection and illness. A low platelet count, called thrombocytopenia, means more likely to bruise and bleed easily.

Some chemotherapy drugs can affect the heart muscle, resulting in cardiomyopathy, or cause arrhythmia. This can affect the heart's ability to pump blood effectively. These problems are less likely to occur if heart is strong and healthy at the start of chemotherapy.

Nervous and Muscular Systems: Chemotherapy drugs may cause problems with memory, or make it difficult to concentrate or think clearly. This symptom sometimes is called "chemo brain." This mild cognitive impairment may go away following treatment, or may linger for years. Some chemotherapy drugs can cause peripheral neuropathy.

Digestive System: Some of the most common side effects of chemotherapy involve the digestive

tract. Mouth sores and dry mouth can make it difficult to chew and swallow. Many patients have a metallic taste in the mouth, or a yellow or white coating on the tongue.

These powerful drugs can harm cells along the gastrointestinal tract. Nausea is a common symptom, and may result in bouts of vomiting. Other digestive issues include loose stools or diarrhea. In some people, hard stools and constipation can be a problem. This may be accompanied by pressure and bloating,. Weight loss and general weakness are common.

Hair, Skin, and Nails: Many chemotherapy drugs affect the hair follicles and can cause hair loss (alopecia) within a few weeks of the first treatment. Hair loss can occur on the head, eyebrows, eyelashes, and body. As troubling as it can be, hair loss is temporary. New hair growth usually begins several weeks after the final treatment.

Some patients experience minor skin irritations like dryness, itchiness, and rash, develop sensitivity to the sun, making it easier to burn. Fingernails and toenails may turn brown or yellow, and become ridged or brittle. Nail growth may slow down, and nails may crack or break easily. In severe cases, they can actually separate from the nail bed.

Sexual and Reproductive System: Some cancer treatments, such as a hysterectomy, cause permanent infertility in women at any age. Another cause of infertility in women is premature ovarian failure, which happens when both ovaries are surgically removed, and may also occur if the ovaries are damaged by radiotherapy or chemotherapy. High-dose chemotherapy is more destructive than lower doses. Chemotherapy with alkylating agents, such as cyclophosphamide, is very toxic and can directly damage the ovaries.

Younger women and those who had lower doses of chemotherapy or radiation therapy are more likely to regain menstrual periods, though they

may not occur regularly. Women over 35 are less likely to recover their fertility. This may be because a woman in her 30s has fewer eggs in reserve, so a larger percentage of eggs are destroyed.

Chemotherapy of the "alkylator" type (such as cyclophosphamide, nitrogen mustard and procarbazine) may cause infertility. The higher the total dose, the more potential for developing infertility. Very high doses can occasionally cause testosterone deficiency. If alkylating chemotherapy was used in combination with radiation, the risk of infertility is increased, and the possibility of testosterone deficiency also exists. Radiation therapy can affect testicular function in two ways: 1) Radiation aimed directly at or near the testicles. The sperm-producing cells (germ cells) are very sensitive to the effects of radiation therapy. Most males who receive radiation to the testicles at doses of 3 to 6 Gy or higher will be infertile. The testosterone producing cells are more resistant to the effects of radiation and chemotherapy, but if testicular radiation was given in doses of 20 Gy) or higher, the Leydig cells may stop functioning, resulting in testosterone deficiency (in addition to infertility). 2) Radiation to the pituitary gland in the brain. Brain radiation can result in damage to the pituitary gland, leading to low levels of the hormones (FSH and LH) needed to signal the testicles to make sperm and testosterone. Surgery that involves removal of both testicles (bilateral orchiectomy) will result in infertility and testosterone deficiency. Pelvic surgery, such as

retroperitoneal lymph node dissection, or spinal surgery sometimes results in nerve damage that may prevent the ejaculation of sperm. Removal of the prostate and bladder may result in difficulties achieving an erection and/or ejaculation.

Kidneys and Bladder (Excretory System): Some drugs are damaging to kidney and bladder cells and can cause acute or permanent renal failure. Some drugs like Ifosphamide cause cystitis.

Skeletal System: Some chemotherapy drugs can cause calcium levels to drop and contribute to bone loss. This can lead to cancer-related osteoporosis, especially in post-menopausal women and those whose menopause was brought on suddenly due to chemotherapy. Women who have been treated for breast cancer are at increased risk for osteoporosis and bone fracture, due to the combination of the drugs and the drop in estrogen levels.

Psychological: Living with cancer and dealing with chemotherapy can exact an emotional toll. Some people may suffer from depression. Juggling work, financial, and family responsibilities while undergoing cancer treatment can become overwhelming.

Financial: Many of the newer agents are more targeted towards the cancer cells and hence have much less toxicity on normal cells .However they are very expensive causing a new class of "Financial Toxicity".

Late Effects of Treatment in Survivors of Childhood Cancer

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Abstract Not Recieved

Effects of Cancer Treatments on Female Fertility

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With improving patient survival, the long term effects of cancer therapies are of increasing relevance, and prominent amongst these is potential loss of fertility. Female fertility can be adversely affected by chemotherapies, radiotherapy and surgical treatment of cancer. The effects of chemotherapy on female reproduction have long been recognised, with immediate effects on growing follicles and fertility, and long term increased risk of premature ovarian insufficiency. Early studies recognised the importance of the alkylating agents as particularly gonadotoxic, and this is very well established. Therapies can generally be classified as high, medium or low risk, but it is important to recognise that most chemotherapy regimens involve multiple agents, and these are evolving all the time. There is

relatively little information on the exact effects of chemotherapeutic agents on ovarian function, but data suggest that different agents can have primary effects on the oocyte, surrounding somatic cells of the follicle, or indeed on the ovarian stroma itself. Radiotherapy also has very adverse effects on the ovary, as the oocyte is highly radiosensitive. The uterus is also sensitive to radiotherapy, resulting in increased risk of miscarriage, prematurity, and low birth weight. Most data suggest that chemotherapy does not adversely affect uterine function. Most interest in relation to surgery and fertility has been on the development of fertility sparing surgery for cervical cancer. Radical trachelectomy has steadily improving results although there remains a significant risk of late miscarriage and premature labour.

Medical Interventions in Protecting Reproductive Functions during Cancer Therapy

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Abstract Not Received

Natural Compounds: Is it a Safe Approach to Protect Gonads from Cytotoxic Agents?

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Due to the increase in childhood cancer survivors the percentage of childhood cancer victims reaching adulthood has increased who may wish to have their family. Under such circumstances the uncertainty over their future fertility potential due to cancer treatment is a matter of concern. Therefore, gonadal tissue protection from the cytotoxic chemotherapeutic agents has become an important issue these days. Since time immemorial natural

compounds have been used in traditional medicinal system based on which, a large number of compounds have been discovered from natural sources today. In this presentation the studies about the application of natural products in protecting the gonadal tissue from cytotoxic agents will be discussed mainly focusing on leaf extract prepared from *Moringaoleifera* plant.

Prepubertal Hematological Malignancies: Practical Approaches in Fertility Preservation

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There has been a significant improvement in cancer survival rates in children over the last decade. As the survival improves, there is increased emphasis on quality of life. Fertility compromise and preservation is becoming an important part of multidisciplinary approach to oncology management in the pediatric population. In children, hematological malignancies are the commonest type of cancers comprising mainly of leukaemias and lymphomas, among which Acute Lymphoblastic leukemia (ALL) is the most common type. Nowadays the treatment is risk adapted i.e., those with higher risk or with advanced stages of disease receive more intense treatment and some of them will end up having bone marrow transplantation. Majority of the cases are curable with drugs and they have minimal effect on the gonadal tissue. Only a few types of lymphomas will require high doses of alkylating agents. One of the consequences of chemotherapy and bone marrow transplantation is damage to the gonadal function, which can lead to loss of fertility. Preservation of fertility before treatment must be considered in all young patients at high risk of infertility and provision of such services requires collaboration between oncology centers and assisted conception units. However, only few methods of fertility preservation can be used in prepubescent boys and girls.

To choose who should we offer fertility preservation (FP) we need to take into account cancer type, the chance of survival, the risk of sub-fertility and the age of the patient. Those having high dose therapy involving alkylating agents or total body irradiation (TBI), those having direct pelvic irradiation, those who are mature enough to understand this and competent enough

to consent this procedure, those who are HIV and Hepatitis B and C negative, should be offered/allowed to choose fertility preservation methods. Whereas we may not offer Gonadal Tissue Cryopreservation to children with an excessive surgical risk such as a bleeding diathesis and where the procedure would cause excessive delay in curative treatment. In cases of known familial genetic predisposition to disease, the patient is a carrier of viral infection (HIV, Hepatitis B or Hepatitis C) as separate storage facilities are required; the decision to do FP needs to be individualized. Psychosocial support should be crucial at the time of diagnosis and whilst offering fertility preservation methods. All necessary information needs to be provided in written to the parents and adolescents.

Treatment options depend on whether the child has gone through puberty. In Prepubescent patients fertility preservation options are limited. Options remain largely experimental such as - ovarian tissue freezing and - testicular tissue freezing. Every effort should be made to limit the radiation exposure by shielding of testes and ovaries should be practiced where possible.

To offer successful fertility preservation to these patients we need to have proper referral process and guidelines with clarity and good team working with coordination of multiple players at all stages. This includes programme and service development (hospital Service), case management (patients, families, oncologists, surgeons, anesthetists, theaters, gynecologists and laboratories). With these experimental methods offered to more and more prepubescent cases it would slowly become the standard management protocol for children with cancer.

First Experience with ANDROPROTECT - A Clinical Program to Preserve Fertility in Boys

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Due to the improved success rates of treating childhood cancers an increasing number of young adults are now the long-term survivors of childhood malignancy. A known late effect of chemotherapy agents and radiation exposure in males is damage to the spermatogonial stem cells in the testis. Thus the treatments used to cure their primary disease may render the patients temporarily or permanently infertile. In this presentation advances in research methods, clinical treatments and patient management strategies used to preserve sperm and testicular tissue for prepubertal boys and adolescents are

presented. The activities of the ESHRE task force on fertility preservation in severe diseases are summarized. This ESHRE group consists of a network of University hospitals providing tissue and cell banking options for young patients. Furthermore many research activities focus on options to create sperm from cryopreserved stem cells. The newly established Marie Curie training program "GROWSPERM" is aiming to implement a number of novel strategies. The current status of clinical and research efforts on male fertility preservation based on spermatogonial stem cells will be reviewed.

Ethical and Social Issues in Fertility Preservation

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Life has two essential goals survival and reproduction. To reproduce and continue to live through our offsprings is the main motto of life. Fertility is a long cherished dream for most human beings; however, there is normally a timeline for this. Nature may have its reasons for restricting the age for fertility, especially in women. Fertility preservation helps bypass this restriction to a certain extent.

Cancer was an incurable illness until the beginning of 20th century. This 'emperor of maladies' was uniformly fatal prior to medical, surgical and radiation oncology treatments. These have added years to the life of these patients. Many of the young cancer survivors regret for having lost their fertility after treatment.

Fertility preservation while offering hope for such patients has also stirred up a lot of ethical concerns.

The advent of cryopreservation has proved to be a boon for the field of reproductive medicine. Cryopreservation was initially done for semen samples, the first report of which was given by Sherman et al, in 1953

1. As the method developed its use progressed to cryopreservation of embryos, and more recently to oocytes also and also the current need of the hour fertility preservation.

Fertility preservation is the cryopreservation of gametes (sperm, oocytes), embryos, testicular tissue and ovarian tissue in order to choose reproduction in the future. This can be for medical

reasons or social (age-related fertility decline)

2. This ingenious technology however raises a lot of ethical issues in each category.

Some of the limitations that apply to cryopreservation in general are:

1. Quality of the biological material cryopreserved
2. Post-thaw recovery/survival rate
3. Variations in protocols across the world

The ethics involved are:

1. Informed consent: for pre-pubertal adolescents with cancer, explaining about the disease and the loss of fertility due to treatment and its consequences
2. Delaying treatment in cancer patients for the sake of fertility
3. Delayed childbearing for social reasons
4. Ownership in case of death, or minors
5. In case of cancer patients, reintroduction of the potential cancerous tissue (ovarian or testicular tissue)

Oocyte preservation: This can be done for social and medical reasons. With multinational companies offering this service to freeze their eggs to its women employees this issue has become more popular raising many debates for and against. The current competitive world pushes the women to be at par with the man in all aspects as a consequence of which and with the choice available, the families are started late. Fertility preservation empowers many women to continue their education and develop their career and financial status, because of the reproductive choice that they have opted. But the biological clock is neither aware of career clock nor the financial status and keeps ticking. It is not certain when she would decide to have the child as career development is akin to chasing the rainbow.

Social indications for oocyte preservations are not as easy as it sounds. It has its own drawbacks.

Few of them are:

1. The protocols of oocyte cryopreservation have not been standardized yet so, the recovery rate is variable and not 100%.

2. The older the women, the quality of oocytes that is frozen is low and therefore the post thaw survival is also low.

3. Fertilization is not absolute neither is pregnancy.

4. Multiple cycles of stimulation for oocyte retrieval and endometrial preparation which has its disadvantages

5. Psychological impact as women tend to delay their pregnancy even further keeping the frozen oocytes as a backup.

Conclusion: Fertility preservation, an essential reproductive technology in the conservation of reproductive function especially in sick patients, serves a very useful purpose. The extension of this technology for social reasons raises several ethical issues. While the issues around fertility preservation in patients on gonadotoxic therapy is limited to ownership of the gametes/ embryos, the ethical issues around social reasons for fertility preservation are surreal and requires greater discussion, more in-depth counseling before it is offered on a commercial scale. We hope it does not become another unrealistic catch phrase, “fertile forever”, as was “feminine forever”, in the last century. One should keep in mind the ground reality while making ethical decisions.

References:

1. Jerome K. Sherman. Historical Synopsis of Human Semen Cryobanking Human Artificial Insemination and Semen Preservation, Springer US 1980, pp 95-105.
2. Stoop, D., Cobo, A., & Silber, S. (2014). Fertility preservation for age-related fertility decline. The Lancet, 384(9950), 1311-1319.

Fertility Preservation in Gynecological Practice: Patient's Perspective

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Fertility preservation is on the rise world over and this rise can be attributed to the increasing ambitions and expectations of people. A few years back, it had gained reputation of being the sole rescue modality for cancer patients but today thanks to the imagination of the ever growing ambition of people to achieve success in their careers, men and women are delaying their parenthood due to work pressure, illness or social situations like divorce or singlehood and preserving their fertility so that they have good chance of becoming a parent when they want.

On one hand, cancer incidence rates in women less than 50 years old continue to increase during recent years, mortality rates are dramatically decreasing due to modern advances in treatment. In 1990 the prevalence of cancer survivors was 1 in 1,000 for young adults (15-45 years of age). By the year 2010, as many as 1 in 250 patients in this age group have survived cancer. However, increasing numbers of survivors are now confronted with the long-term consequences of exposure to these treatments, which includes surgery, radiotherapy, and chemotherapy. These modalities have a profound impact on ovarian function, leading to premature menopause and loss of fertility.

Given that the pool of primordial follicles in the ovary is fixed and declines in a predictable manner, generalized models have been established to describe the natural decay of the ovary. Any injury to the ovary can potentially reduce this ovarian reserve, effectively advancing the patient's reproductive age, thus closing her window of reproductive opportunity. Another interesting yet disturbing fact to be worth discussing is, in this era of cut throat competition, the concept of buying time to enhance career options and postpone pregnancy has brought egg freezing into mainstream. Instead of waiting for the right time to put their careers on hold, or wondering whether a great partner and potential father might come into their lives, women are seeing egg freezing as a way to buy time, and this trend is seen catching up with males too!! Recently Apple and Facebook made headlines by adding egg freezing to their roster of employee benefits, and it's expected more will follow suit.

The appeal of egg freezing is clear: When it comes to something as important as the decision to have children, why not invest in a little insurance for the future?

Strikingly, one of the world's leading reproductive-technology associations warns against using it as a way to preserve a woman's fertility beyond her natural reproductive years. While the American Society for Reproductive Medicine (ASRM) lifted the "experimental" label, it warns in the same document that because so little is known about the potential risks and long-term impacts, egg freezing should not be routinely offered to women worried about their future fertility.

Where one finds it as a boon for cancer patients, Federal and provincial oversight of assisted reproductive services is largely non-existent. As a result, clinics are left to their own devices as to how they market egg freezing, who qualifies for services, what they charge and what they tell potential customers. One can thus believe, that the lack of oversight means "it's only probably a matter of time" before serious problems emerge.

Recent advances in cancer therapy have resulted in increasing numbers of long-term survivors who are then left to deal with the consequences of their treatments. The treating physician should take an active role in both reducing potential ovarian toxicity in every way possible and in providing the means whereby the patient can make an informed decision regarding her options for fertility preservation. Ongoing research in fertility preservation shows promise for those female cancer survivors who face POF and infertility. Patient-to-patient variability is problematic and better definitions of outcomes and controlled trials of post-therapy management are warranted.

Thus one can conclude that selection of the right modality to right patient, can help use this boon for the betterment of those who desire pregnancy. It's also very important for women who do it to realize it does not guarantee them a baby. It only provides the hope of a baby"

Informing Patients about Fertility Issues Before Cancer Therapy

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Improvement in cancer treatment has resulted in increased survival, which makes late term side effects like gonadal failure very important. Both neoplastic disease and its treatment interfere with sexual and reproductive function. As with other potential complications of cancer treatment, oncologists have a responsibility to inform patients about the risk that their cancer treatment will permanently impair fertility. Individual factors such as disease, age, treatment type and dosages, and pretreatment fertility should be considered in counseling patients about the likelihood of infertility. Patients who are interested in fertility preservation should consider their options as soon

as possible to maximize the likelihood of success. It is also important to emphasize the fact that there appears to be no detectable increased risk of disease recurrence associated with most fertility preservation methods and pregnancy, even in hormonally sensitive tumors. Also patient to be told that there is no evidence that a history of cancer, cancer therapy, or fertility interventions increase the risk of cancer or congenital abnormalities in the progeny. Patients also have to be educated that chemotherapy induced infertility is temporary in many cases and restoration of fertility usually occurs within few months after chemotherapy is completed.

Role of Oocyte Vitrification for Fertility Preservation Programs

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Fertility preservation is an emerging, rapidly evolving branch of reproductive medicine comprising the preservation of gametes (sperm, oocytes) and reproductive tissue (ovarian, testicular), giving individuals at risk of losing their reproductive ability the chance to conceive and have their own genetic offspring. This presentation is focused in the oocyte vitrification as fertility preservation approach.

After more than two decades of continuous efforts made to improve cryopreservation techniques, the introduction of vitrification into assisted reproduction have achieved efficient and reproducible protocols to successfully cryopreserve the female gamete.

Fertility preservation has being used routinely for relatively few years. Cancer patients who are to undergo surgery or start chemotherapy or radiotherapy, women with other medical conditions leading to premature menopause but

also healthy women wishing to postpone childbearing are the main beneficiaries of this strategy.

At present we have a large database from ovum donation programs and many healthy children have been born from cryopreserved oocytes for non-medical (social) indications. However, available information about cancer patients who have achieved pregnancy with their own cryopreserved oocytes is still limited.

Using data from IVF, Spain, the present work aims to show the clinical use of oocyte vitrification in the fertility preservation context for non-oncologic and oncologic patients. Likewise, the obstetric outcome of first pregnancies achieved after vitrification and warming of oocytes from women being treated for cancer is also analyzed.

Ovarian Preservation in Surgical Management of Gynecological Cancer

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Abstract Not Recieved

Laparoscopic Cystectomy in Benign Ovarian Condition and Ovarian Preservation

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The presentation will focus on an important issue whether laparoscopic cystectomy procedure leads to removal of normal ovarian parenchyma in relation to the size of the cyst. Prospective study of 56 women attending University teaching hospital who underwent laparoscopic endometriotic cystectomy from August 2010 to August 2011. All patients underwent Transvaginal sonography before surgery. Largest transverse diameter of cyst was noted. Laparoscopic surgery was performed by four puncture technique. Endometriosis was staged according to revised American Society for Reproductive Medicine classification (rASRM). Cystectomy was done by stripping technique, sent for histopathological evaluation, and was graded based on semi quantitative scale of 0-4. 56 women were categorized into 2 groups. Group 1 showed grade 0, 1, 2 and group 2 showed 3, 4 in the cyst wall. Mean age of patients was 31.4 (20-41 years),

mean duration of infertility was 4.1 (2-18 years) and mean cyst diameter measured 6.3 cms (1.8-7.5). 41 women were in group 1 indicating no ovarian parenchyma loss and 15 were in group 2 indicating significant loss of ovarian tissue, mean cyst diameter being 4.3 cms and 5.0 cms respectively. There was no significant statistical correlation between preoperative cyst diameter and ovarian parenchyma removed ($p=0.15$). In 15 women of group 2, 14 were found to have moderate to severe endometriosis. In 41 women of group 2, only 27 showed severe endometriosis, indicating there is correlation between disease severity and loss of ovarian tissue ($p=.04$). In conclusion endometriotic cystectomy when performed with accurate surgical technique leads to no significant ovarian tissue removal. However disease severity significantly determines the loss of normal ovarian parenchyma

Current Methods of Fertility Preservation

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The great improvement of survival rates in malignancies experienced over the last 20 years in has changed the concept to that oncologists now not only aim to cure the neoplastic conditions but also to avoid/minimize the side effects of the treatment used. In this context, fertility preservation is a central point, since infertility is

one of the most well-known side effects of chemotherapy and radio-therapy. The aim of this lecture is to identify the available options for fertility preservation in women, with risk of gonadotoxic side effects of cancer treatment, and to describe their main particularities in order to maximize clinical success.

The groups of women that can benefit from ovarian stimulation protocols with subsequent IVF with embryo cryopreservation or oocyte freezing will be identified. Short stimulation protocols that can be started in follicular or luteal phase will be described.

Ovarian tissue cryopreservation and transplantation has become a valuable option for fertility preservation, especially for patients who cannot benefit from other preservation techniques such as ovarian stimulation. In the present lecture these topics will be covered: 1) to build a body of rules that allow for an easy identification of potential patients susceptible to undergoing ovarian tissue cryopreservation/transplantation,

2) to set out the evaluation and management of such patients before cryopreservation, before and after re-transplantation of the cryopreserved ovarian tissue, 3) to explain the technical aspects of ovarian tissue transplantation, from laboratory procedures to surgical tips and clinical management after transplantation, 4) to summarize the main limitations of ovarian tissue transplantation, to describe the different strategies proposed to overcome such limitations and to give an overview of the efficacy of this procedure.

In conclusion, there exist several alternatives for fertility preservation in women and the available options will most likely increase further in the future.

Patient Selection for Fertility Preservation

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Many patients will retain fertility after cancer treatment, whereas others are at high risk of becoming infertile. It is therefore important to try and identify those at particular risk, so that fertility preservation options can be discussed with them, and if appropriate, taken forward. Risk can be classified according to diagnosis, where it is important to recognise that it is, of course, the treatment rather than the diagnosis that results in the loss of fertility. Chemotherapy agents can be classified by risk, with the alkylating agents recognised to be the most gonadotoxic. Pelvic radiotherapy, or abdominal radiotherapy in children, also carries a high risk to future fertility, and certain surgical procedures will carry obvious risks, for example in the treatment of cervical cancer. We have proposed a framework for assessing risk to the patient, based on dividing issues into those that are intrinsic to the patient, and those that are extrinsic. The intrinsic factors include the health of the patient, her pubertal status, and assessment of the ovarian reserve. Extrinsic factors include, most importantly, the

nature of the predicted treatment, the time available, and, to an extent, the practicalities involved such as the expertise and options actually available to her. The options include ovarian tissue cryopreservation, oocyte vitrification, and embryo cryopreservation. The latter commits her oocytes to fertilisation, which will not be appropriate for many women of the ages being considered, and both oocyte and embryo storage require ovarian stimulation which is not appropriate in young girls. While these two procedures are, however, increasingly routine, ovarian tissue cryopreservation remains an experimental procedure, particularly in children. Patient age is the most established index of ovarian reserve, but there is increasing data that precancer treatment AMH may also be useful in predicting the likelihood of post treatment ovarian function. Using this approach, it may be possible to distinguish between patients at high risk of loss of fertility, for whom intervention may well be appropriate and those where fertility is likely to be retained.

Human Ovarian Tissue Vitrification in Oncofertility Practice

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Improved long-term survival rates of cancer affected women along with advances in reproductive medicine and cryobiology have increased the interest in fertility preservation methods in these patients. Among these, ovarian tissue cryopreservation has emerged as a promising option to safeguard fertility of prepubertal girls or patients who need immediate chemotherapy. To date, autotransplantation of ovarian cortical fragments cryopreserved through slow-cooling has resulted in 30 live births. In spite of recent progress in slow cooling procedures, many authors have investigated vitrification as an alternative option for cryopreserving ovarian tissue. Vitrification procedure is influenced by many variables, such as type, exposure time and concentrations of cryoprotectants (CPAs), sample size, carrier systems used, cooling rates and also technical expertise. Thus, in the last few decades, different approaches have been applied to improve vitrification efficiency. Several devices have been

used to minimize the volume of CPAs and increase cooling rates, such as Cryotop, electron microscopy grids, Cryoloops and quartz capillaries. Moreover, use of slush nitrogen (SN2) vitrification has been demonstrated to improve the cryopreservation of human spermatozoa, mouse and ovine oocytes, mouse and murine embryos and also clinical efficacy of human oocyte vitrification. Our team recently demonstrated that SN2 vitrification allows a better preservation of ovarian tissue, favouring: 1) the recovery of a higher percentage of morphologically intact follicles; 2) an improved preservation of follicular and stromal cells shown at an ultrastructural level and 3) a better maintenance of DNA integrity, avoiding DNA fragmentation after thawing. These encouraging results open new interesting perspectives in fertility preservation procedures and may lead to future application of vitrification for routine ovarian tissue cryopreservation in clinic.

Ovarian Cryopreservation in Sweden: Clinical Results and Research Efforts

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Ovarian cryopreservation was established in two centres in Sweden (population 9.5 million) already in 1998. In the beginning various cryopreservation protocols were used. The most well-structured program for fertility preservation in Sweden during the last decade has been that of Karolinska University Hospital in Sweden. In this lecture the characteristics of the patients that have undergone ovarian cryopreservation in Sweden will be described. A

small number of retransplantation attempts have been made and in 2013, the first live birth occurred from a patient of the Karolinska Hospital cohort. This was from a woman with Hodgkin's disease, who had been treated with both chemotherapy and radiation therapy. Her uterine size was decreased. In conclusion, the lecture will give a summary of the Swedish efforts and clinical success in ovarian cryopreservation.

Strength and Weakness of a Testicular Stem Cell System in Man during Normal and Disturbed Gonadal Function

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Spermatogonial stem cells open novel strategies for preservation of testicular tissue and fertility preservation in boys and men exposed to gonadotoxic therapies. This talk will provide an update on the physiology of spermatogonial stem cells in rodent and primate testes. Species-specific differences must be considered when new technologies on testicular stem cells are considered. Male infertility may be related to defects in spermatogonial stem cells or the stem cell niches. Loss of germ cells may be a consequence of germ cell quality assessments as checkpoints are passed prior to entry into the spermatogenic process. Curative options for spermatogonial stem cells in man are not yet

established. Germ cell transplantation is presented as one strategy. Whereas this technique has become an important research tool in rodents, a clinical application must still be regarded as experimental. Grafting of immature tissue fragments revealed a high regenerative potential of immature testicular tissue. Grafting was applied in rodents and primates and resulted in the generation of sperm. In vitro cell or organ culture has also been tested and recently showed promising results at least in mouse models. The advantages and challenges of all techniques will be visited and potential future strategies for expansion and differentiation of male germ cells are discussed.

Uterus Transplantation to Restore Fertility after Cervical Cancer Surgery

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The last frontier to conquer in female infertility is absolute uterine factor infertility (AUI), affecting around 200,000 women in Europe. A small group of women with AUI are those affected by malignancies of the uterus or uterine cervix. Since cervical cancer affects women from the age of 20, this malignancy will be the most common direct cause of AUI.

Uterine transplantation (UTx) is now the first available treatment for this large group of women. Adoption and gestational surrogacy are other means to obtain motherhood, but the acceptances of these arrangements in the society vary greatly between societies.

Our research group initiated a step-by-step developmental animal-based research approach on UTx in 1999 and have optimized all aspects of the procedure in several animal species. Today 11

human UTx attempts have been made, with the last 9 of them performed by our team. The first two human UTx-attempts were done in Saudi Arabia in 2000 and in Turkey in 2011. Both these teams had no research experiences in the field. These two transplantations have not been successful.

In early 2013 our team completed the surgeries of a series of totally 9 human UTx, with live uterus donors. Eight recipients were MRKH patients and one had been hysterectomized because of cervical cancer. The cervical cancer patient (squamous cell carcinoma, Stage 1b) was the first to be operated. She had undergone a radical hysterectomy with pelvic lymphnode dissection with preservation of the ovaries 7 years prior to transplantation.

The mean age of the recipients was 31.5 +/- 3.9 years. Five donors were mothers and this included the donor of the cervical cancer patient. Other

donors were close relatives and in one case family friend. The mean age of the donors was 53.0 +/- 7.0 years. IVF treatments were done before transplantation. The donor surgery involved uterine isolation with pedicles of the uterine arteries and veins and including large parts of the internal iliacs. The duration of these surgeries were between 10h 17min and 13h 8min (11h 37 min + 1h 54 min). No donor needed perioperative blood transfusion and the hospital stay was 6 days. One complication occurred, with aorto-uterine fistula in donor #2 two weeks after surgery and repaired 3 months later.

In the recipient a midline incision was used and the external iliac artery and vein were mobilized bilaterally. Bilateral end-to-side anastomosis was accomplished between the uterine artery and one major uterine vein on each side, using 7-0 and 8-0 sutures on arteries and veins, respectively. After commencement of uterine perfusion the vaginal-vaginal anastomosis was accomplished. The graft was fixed to the round, cardinal and sacrouterine ligaments and an extensive leaf of bladder peritoneum of the graft was sutured on top of the bladder for extra structural support. The duration of recipient surgery operations varied between 4h 10min and 5h 56min (4h 46min + 30min). None needed perioperative blood transfusion and the hospital stay varied between 3 and 9 days. The recipients received two ATG treatments perioperatively and corticosteroids for 4 days. They were then only on double

immunosuppression with tacrolimus and MMF and the plan was tapered doses of tacrolimus and omission of MMF after 6 months, to avoid possible teratogenic effects of MMF.

Two patients had to be hysterectomized during the initial months due to uterine complications. In recipient #2, an intrauterine infection (*Enterococcus faecalis*) was diagnosed 33 days after UTX and despite repeated attempts with iv antibiotics and surgical drainage the infection progressed with septic symptoms necessitating hysterectomy 105 days after UTX. In recipient #9 (heterozygote for the Leiden mutation) uterine artery thrombosis was diagnosed on the 3rd postoperative day and a non-perfused uterus was removed.

Mild rejection episodes have occurred in 5 of the seven successfully transplanted patients and all have been reversed by 7-10 days of corticosteroid treatment. All these seven patients have shown regular menstruations from 2 months after UTX. Embryo transfers have started in all 7 patients during the spring of 2014. The first livebirth after UTX was occurred in September 2014. The cervical cancer patient got pregnant at the 5th ET attempt and the pregnancy has evolved uneventful up to more than 35 weeks.

The successful pregnancy after UTX in a cervical cancer patient is a proof-of-concept of UTX as a method of fertility restoration after hysterectomy for uterine/cervical malignancy.

Embryologist's Perspective in Handling Gametes of Cancer Survivors in ART

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There have been positive and dramatic innovations and awareness in the overall diagnosis and treatment of cancer patients. The impact of these have been on the survival rates of cancer patients. Growing knowledge of oncological pathophysiology, individualizing of cancer therapies, less radical and more conservative approach and advances in ART have contributed in fulfilling the desire of cancer patients towards

leading a normal life with the possibility of parenting their own children. The ASCO recommendations suggest that fertility preservation is of great importance to many patients diagnosed with cancer and infertility resulting from cancer treatment may be associated with psychological distress. Chemotherapy and radiotherapy can affect the fertility of the individual and some of the

contributing factors here are drug, dose, size and location of the radiation field, intensity method of administration (oral or intravenous) etc.

From an embryologists point of view the ideal option on gamete cryopreservation would depend on recovery and efficacy of the procedure, storage and its validity, security and probability of contamination of the stored samples and procedural changes that may occur in the future. His major constraint is the knowledge that there would be no second sample available and no option of selecting or deselecting the gametes, whatever is available today may be the only promise for the patient's fertility.

Male gamete: In the male the compromised fertility can be accessed by means of a basic semen analysis which will show a reduction in the count, motility and morphology. The DNA integrity may also be compromised. Sperm cryopreservation is a well established technique even in samples with reduced count and motility. These sperm can be collected by masturbation, or surgically from the testis. There have also been case reports of sperm collection from the urine sample post masturbation and by recto ejaculation under sedation or anaesthesia.

Sperm banking is recommended before initiation of the treatment. Some cancers particularly testicular and Hodgkin's lymphoma can affect the sperm quality adequate number of ejaculates should be stored. ICSI offers a very promising option for these patients to father their own children. Freezing of testicular tissue or germ cells and reimplantation of this tissue post cancer treatment has been successfully applied in animal models but have not been attempted in humans.

Female gamete: In the female there can be an altered hormonal balance, reduced number of primordial follicles, surgery and radiation can distort the anatomy of the reproductive tract and also compromise its vascularity. Chemotherapy and radiation specifically to the pelvic region can lead towards POF.

Ideally, embryo CP would be the best and most successful means of fertility preservation in females. However the option depends on the patients age, time available to initiate cancer therapy and whether she has a partner available at

the time of treatment. As some cancers like oestrogen positive breast cancer are hormone dependent and hence stimulating these patients for the purpose of obtaining more oocytes followed by either freezing them as oocytes or embryos through IVF may be contraindicated. However letrozole (where approved) and tamoxifen can be used safely for stimulation of the ovaries. Oocyte cryopreservation is now no longer considered 'experimental' and there is enough data reassuring us of the success of this procedure. In fact, there have been few reports of live births in patients with cancer who opted for oocyte cryopreservation. In patients where a partner is not available or those with religious or ethical limitations to embryo freezing, oocyte freezing can be offered.

In vitro maturation of oocytes along with vitrification can also be considered as another option. The oocytes can be obtained either by stimulating the ovaries or in a natural cycle where stimulation of ovaries is unsafe or where the cancer therapy cannot be delayed. As the GV structurally may avoid spindle depolarisation during freezing it is believed that the immature oocytes can be vitrified and post thaw allowed to mature. However the potential of the oocyte to mature in vitro post vitrification and warming seems to be reduced significantly suggesting that vitrifying mature oocytes would be a better option.

Ovarian tissue cryopreservation has been considered primary method of fertility preservation in prepubertal girls along with transplantation later after puberty. Ovarian tissue cryopreservation along with immature oocyte collection followed by IVM-vitrification can also be considered prior to gonadotoxic treatment.

Preliminary Studies at Fertility Preservation - An Indian Experience

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The most important issue for young cancer patients is FERTILITY PRESERVATION. The life expectancy of such patients have been greatly enhanced by aggressive chemo and radiotherapy. But these treatments are cytotoxic to the gonadal tissues resulting in infertility.

This is a retrospective study done at Gunasheela IVF Centre from November 2010 to February 2015. 135 males have had their sperm cryopreserved 39 were married & 96 were unmarried. Average age of these patients was 26 years (16-39 yrs). Commonest indication was Testicular Cancers (57), followed by Hodgkin's Lymphoma (25), miscellaneous (36), Leukemia (6) and other lymphomas (6).

19 women who opted for fertility preservation were

of average age 26 (16-39). 11 were married and 8 were unmarried. Commonest indication was Breast Cancer in 9 patients, Ovarian Cancer in 4 patients, Hodgkin's in 2 patients, Miscellaneous in 3 patients. In women, oocytes, embryos, ovarian tissue or combinations of these were frozen. Two of these patients have conceived spontaneously and delivered full-term normal babies. Only 1 patient has come back as yet for Frozen Embryo Transfer this month.

Fertility preservation is an integrated approach involving onco-surgeon and infertility specialist. Counselling plays an extremely important role in this treatment. This study needs long-term follow up of these patients to see the outcome and arrive at conclusions.

Restoring Fertility in Cancer Survivors: Ovarian Tissue Transplantation or ART?

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Advancements in cancer therapies, particularly chemotherapeutics, have led to dramatic improvements in survival. The American Society of Clinical Oncology in 2006 recommended Oncologists to discuss fertility preservation with all patients of reproductive age (and with parents or guardians of children and adolescents) if infertility is a potential risk of therapy and to refer patients who express an interest in fertility preservation to reproductive specialists.

Embryo cryopreservation is an established fertility preservation method, and it has routinely been used for storing surplus embryos after in vitro fertilization. Cryopreservation of unfertilized Oocytes is an option, particularly for patients who do not have a male partner, do not wish to use donor sperm, or have religious or ethical objections to embryo freezing. As of October 2012,

the American Society for Reproductive Medicine no longer deems this procedure experimental.

Ovarian tissue cryopreservation for the purpose of future transplantation does not require ovarian stimulation or sexual maturity and hence may be the only method available in children. It is considered experimental and should be performed only in centers with the necessary expertise that include follow-up for recurrent cancer. Although this process is still considered experimental, successful pregnancies have been reported.

Other options like ovarian transposition (oophoropexy) when pelvic radiation therapy is performed as cancer treatment and conservative gynecologic surgery and radiation therapy should also be discussed.

Patients should be informed that there is insufficient evidence regarding the effectiveness of

ovarian suppression (gonadotropin-releasing hormone analogs) as a fertility preservation method, and these agents should not be relied on to preserve fertility.

Time is of the essence. Fertility preservation treatments need to be completed before the start chemotherapy and/or irradiation.

In particular, no patient should be excluded from

consideration for discussion of fertility preservation for any reason, including age, prognosis, socioeconomic status, or parity. Discussing infertility and introducing the possibility of fertility preservation leads to improved quality of life and diminished distress in all patient populations.

Ovarian Stimulation in Cancer Patients: Approach and Challenges

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There are approximately 180,000 new patients with breast cancer each year in the United States alone, and 15% of these women are of reproductive age. Consequently, at least 25,000 women may potentially suffer from ovarian failure and infertility because of exposure to gonadotoxic chemotherapy each year.

Most combination chemotherapy regimens include the alkylating agent cyclophosphamide, which is known to cause a significant loss in ovarian follicle reserve. This diminishment in ovarian reserve results in premature ovarian failure and infertility with serious consequences to the quality of life. Although ovarian tissue and oocyte freezing are still experimental, embryo cryopreservation is an established clinical procedure after in vitro fertilization (IVF). However, IVF typically requires ovarian stimulation with a resultant increase in estrogen levels. A high-estrogen milieu is not considered safe for breast cancer patients. Clinical studies in which letrozole (antiestrogen) was typically administered at doses of 2.5 to 5 mg for 5 days have also shown its benefit in ovulation induction alone or in combination with follicle-stimulating hormone (FSH). These studies also showed that peak E2 levels were lower when letrozole, alone or in combination with FSH, was compared with stimulation with FSH or clomiphene. E2 levels have been found to be even lower than in natural cycle in patients stimulated with letrozole. Because letrozole can induce ovulation without raising

estrogen levels, there is a hypothesis that it can be safely used in patients with breast carcinoma undergoing IVF. Tamoxifen has been tried for ovulation induction.

Since there should not be any delay in starting the ovulation induction, antagonist protocol is preferred. The target should be to get about 15-20 follicles. Chances of Ovarian hyper stimulation syndrome (OHSS) is rare as there will not be Embryo transfer.

Cancer is associated with increased catabolic state which may affect HPO Axis leading to increased dosage of the ovulation inducing drugs.

If the patient is in the luteal phase of the menstrual cycle at the time when the option for ovarian stimulation is made for subsequent oocyte or embryo cryopreservation, the treatment may extend over a total of up to six weeks which, in some cases, is unacceptable due to the need for early chemotherapy and/or radiotherapy. There are situations in which, due to the imminent necessity of starting a cytotoxic treatment, it is not possible to schedule the beginning of ovarian stimulation during the early follicular phase of the menstrual cycle or after pituitary blockade with GnRH agonists. In view of these considerations, a possible perspective is to consider the possibility of performing ovarian stimulation even in the luteal phase of the menstrual cycle, as long as the pituitary is blocked with a GnRH antagonist 3 to 4 days before the procedure or concomitantly with the beginning of ovarian stimulation with

gonadotropins. Hence, oocytes may be obtained before the beginning of cancer treatment regardless of the phase of the menstrual cycle during which ovarian stimulation is started.

One study(1) showed that the stimulation was performed with highly purified uFSH 300 IU/day and 0.25 mg/day GnRH-antagonist starting on cycle day 19-21 of a spontaneous menstrual cycle and commencing until hCG administration when three follicles ≥ 17 mm were present. Mean stimulation duration was 11.7 (SD 1.6) vs. 9.1 (SD 1.3) days, mean cumulative FSH dose was 3,495.0 (SD 447.5) vs. 2,040.5 (SD 576.2) IU, and mean number of oocytes was 8.8 (SD 5.0) vs. 10.0 (SD 5.4) in study vs. control group, respectively. Per follicle ≥ 10 mm, the cumulative FSH dose was 274.5 (SD 130.8) IU vs. 245.2 (SD 232.3) IU in study and control groups, respectively. Cumulative ongoing pregnancy rates were 1/10 (10 %) and 6/30 (20.0 %) in study and control group, respectively (difference: 10 %, 95 % confidence interval of the difference: -29.2-22.2 %, $p = 0.47$). Fertilization rate was similar between groups, with 63.5 % (SD 32.9) in the study and 61.3 % (SD 26.7) in the control group, respectively. Serum estradiol levels were significantly lower on the day of triggering final oocyte maturation with 1,005.3 (SD 336.2) vs. 1,977.4 pg/ml (SD 1,106.5) in study and control group, respectively. Similarly, peak estradiol biosynthesis per growing follicle ≥ 10 mm was lower in the study group (134 pg/ml, SD 158.4 vs. 186.7 pg/ml, SD 84.7). Per retrieved oocyte, a nearly threefold higher dose of FSH had to be administered when ovarian stimulation had been initiated in the luteal phase. Furthermore, the present study casts doubt on the efficacy of initiating ovarian stimulation in the luteal phase in terms of pregnancy achievement.

Ovarian stimulation starting in the luteal phase of the menstrual cycle concomitantly with pituitary blockade with a GnRH antagonist lasted on average seven days, a fact that did not cause postponement of the beginning of the recommended chemotherapy. Hence there are studies which suggests that oocytes can be obtained before cancer treatment efficiently irrespective of the phase of the menstrual cycle. Ovarian stimulation was initiated with hMG and

letrozole 2.5 mg daily after spontaneous ovulation can also be tried(2). Letrozole administration was stopped when the dominant follicles reached diameters of 12 mm. This also yielded good oocytes for further use. Of the 242 women enrolled in the study, all participants succeeded in producing oocytes and 227 women had highest-quality embryos to cryopreserve. The average number of oocytes retrieved was 13.1, producing an average of 4.8 highest quality embryos. Moreover, no cases experienced a premature LH surge or moderate/severe ovarian hyperstimulation syndrome during the stimulation cycles. In FETs, the clinical pregnancy rate, ongoing pregnancy rate, and implantation rate were 55.46% (127/229), 48.91% (112/229), and 40.37% (174/431), respectively. Of all the pregnancies in the study, 68 resulted in live births and 44 were ongoing. Patients were submitted to ovarian stimulation with recombinant follicle stimulating hormone together with pituitary blockade with a GnRH antagonist during the luteal phase of the cycle. Oocyte retrieval was performed nine days after the beginning of ovarian stimulation, with 12 mature oocytes being obtained in both cases. In case 1, all mature oocytes were submitted to ICSI, with fertilization and cleavage rates of 83.3% and 70%, respectively, and with the formation of seven good quality embryos. In case 2, all of mature oocytes were cryopreserved. The study concluded that these cases demonstrate that it is possible to obtain mature oocytes when ovarian stimulation is started in the luteal phase in situations in which there is not sufficient time for conventional stimulation. There are other studies supporting the luteal phase ovarian stimulation and also the Random start controlled ovarian hyperstimulation with letrozole for fertility preservation in cancer patients(3)(4).

Conclusion: In ovarian stimulation for ART prior to chemotherapy, a high dose of Gonadotropins need to be used along with antagonist. However stimulation can be started at any phase of menstrual cycle since GnRH antagonist can suppress LH surges.

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Semen Cryopreservation in Fertility Preservation Program

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Sperm cryopreservation is a routine procedure successfully performed in ART setup since the refinement of the technique in 1950. The most common indications are a) to avoid inconveniences due to failure to produce semen sample on the day of IVF procedure b) sperm banking used in heterologous insemination. However another group of patients in whom sperm cryopreservation becomes particularly important are people diagnosed with cancer. Cryopreservation of sperm sample prior to initiation of cancer treatment or during the treatment is strongly advised as chemo or radiotherapy can lead to testicular failure.

Rapid freezing of spermatozoa proposed by Sherman in 1990 is the most successful method of sperm cryopreservation. This technique offers considerable success in cases of high number of spermatozoa, which may not be always the case in cancer patients. There are plenty of reports in the literature suggesting adverse effects of cancer on sperm quality, though the exact reasons for this are unknown. In such conditions novel freezing approaches such as freezing in empty zona pellucida, capillaries and cryo loops have been tried. The potential negative effects of cryopreservation on sperm motility, morphology,

viability and fertilising ability are well documented. There is also a possible damage to sperm DNA post cryopreservation. It has been shown earlier that poor quality spermatozoa are more vulnerable to freeze thaw damage. To reduce the damage several modifications to the freezing technique such as addition of seminal plasma, preparation of semen sample prior to cryopreservation and addition of motility enhancement drugs have been proposed. Recently cryoprotectants free vitrification of spermatozoa is gaining importance as a more simpler and faster alternative to conventional cryopreservation techniques. Though sperm cryopreservation has emerged as an integral part of fertility management in adolescent cancer survivors, at present this technique is under utilised due to lack of awareness. Optimising freeze thaw protocols and minimising damage during cryopreservation by the right choice of carrier and cryoprotectants would improve cryo survival rates and increase fertility options in cancer survivors. Technical aspects of sperm freezing and modifications to the technique shall be discussed in detail.

Avoiding the Transmission of Malignant Cells: Single Culture of Primary Follicles versus Culture of Ovarian Slices

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Recent advances in cancer therapy has, tremendously increased the life expectancy in cancer affected women. Most anti-cancer regimes adopted today including chemo and radiotherapy threatens the reproductive future of cancer survivor's. Ovarian tissue cryopreservation has gained great acceptance as a promising fertility preservation strategy in recent years. However, recent research and further analysis on the safety of this procedure has led to concerns of re-introducing malignant cells back into the cured patient. Hence, in patients with systemic malignancies like leukaemia, or other tumours with high chances of metastasis to the ovaries, ovarian tissue cryopreservation is presently not advisable. To circumvent this issue, culture systems to maintain follicles in vitro and possibilities of transplanting isolated follicles that do not transmit cancer affected cells are being considered. Although, attempts to culture single follicles within a defined physical and chemical environment have been tried in this regard, failure to completely mimic the in vivo follicular environment has encouraged the culture of follicles within ovarian cortical slices, allowing growth within their natural environment. Optimizing early follicular culture and recruitment in vitro, could greatly enhance current fertility preservation strategies, by offering a larger population of functionally competent gametes to the patient without the added risk. This could in turn ensure the efficiency and long term safety of these fertility restoration efforts.



POSTER PRESENTATIONS



P1

Protective Effect of Vernonia Cinerea on Cisplatin Induced Testicular Damage in Mice A Pilot Study

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Cisplatin is a potent antineoplastic drug and the clinical usage is limited due to dose related side effects. Male reproductive system is one of the targets of cisplatin induced toxicity. The toxicity includes alteration in structure and functions of Sertoli and Leydig cells, germ cell apoptosis and increase in microtubule stability. Vernoniacinerea (VC) is a small herb, used in Siddha system to reverse drug induced toxicities. Many studies have supported the protective effect of VC against cisplatin induced organ toxicities. No studies have been done to evaluate the protective effect of VC on cisplatin induced testicular toxicity. Hence, the pilot study was conducted. A total of 12 male albino mice were divided into four groups containing three mice in each group. Animals in Group 1 served as normal control and the testes were not damaged. Testicular damage was induced in groups 2, 3 and 4 by administering a single dose of cisplatin (5.5mg/kg, i.p) on day 4. Group 2 did not receive VC extract treatment. Group 3 and 4 received 400mg/kg dose of aqueous and ethanolic extract of VC respectively from day 1 (3days prior to

cisplatin) until day 15 (11 days after cisplatin). After 15 days of treatment with VC extracts, the testes were collected for haematoxylin& eosin staining. The histology slides were observed under microscope and qualitatively compared between different groups. The qualitative microscopic observation of group 1 showed normal histological appearance. Cisplatin treatment in group 2 induced maturation arrest, mild interstitial edema, reduced spermatogenesis, tubular atrophy, loss of spermatogonia, and germ cell apoptosis with karyorrhexis. VC extracts did not reverse interstitial edema, tubular atrophy and loss of spermatogonia. But, it showed mild focal reversal of maturation arrest and reduction in cisplatin induced germ cell apoptosis. Fifteen days administration of Vernoniacinerea extracts has shown mild protective effect against cisplatin induced seminiferous tubule toxicity. Currently, the work is being continued in an effort to identify the active principle responsible for the protective effect against cisplatin induced toxicity.

P2

IL2 and IL41I in Women with Recurrent Pregnancy Loss

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The American Society for Reproductive Medicine has defined recurrent pregnancy loss(RPL) as two or more failed a fetus up to 20 weeks of pregnancy and weighing up to 500 g. The exact etiologies for the recurrent pregnancy losses have not been clearly explicated, and time and again remain undefined. Recurrent pregnancy loss and recurrent implantation failure are mostly associated with immunological factors mainly due to abnormal NK cell activity. Immunological factors regulate NK cell proliferation, differentiation, and production of

cytokines and other molecules that support placental and trophoblast development and promote local immunomodulation.IL-2 is capable of inducing proliferation and augmenting cytotoxic activity in NK cells which can adhere to and lyse human endothelial cells.IL41I reduced inflammation associated with the control of the infection and has evolved from ancestral innate antimicrobial functions to acquire a regulatory effect on the adaptive immune system such as NK cells. The mechanism is IL-2 mediated inhibition of

the IL-4 induced secretion. Therefore the objective of this study was to investigate the serum levels of interleukin 2 (IL2) and interleukin 4 induced 1 (IL4I1) in patients with recurrent pregnancy loss and to correlate with healthy women as well as women in the 1st trimester of pregnancy. Fifty patients with history of recurrent pregnancy loss undergoing a treatment were enrolled into the study. The serum IL2 and IL4I1 of patients were analyzed using ELISA, and compared with that of 40 healthy volunteers as well as women in the 1st

trimester of pregnancy. In patients (n=50) serum level of IL4I1 was found to decrease with a $p < 0.0001$ when compared with healthy women and women in the 1st trimester of pregnancy but IL2 was found to be highly increased with a $p < 0.0001$ when compared with control both control groups. We therefore conclude that IL4I1, IL2 and copper plays an important role in recurrent pregnancy loss. They might be observed as predictors for miscarriage and the efficacy of treatment.

P3

Predictive Value of Follicle Stimulating Hormone (FSH), Anti-Mullerian Hormone (AMH) and Antral Follicle Count (AFC) on the Oocyte Yield in IVF Cycles

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Anti-Mullerian hormone as a predictor of ovarian reserve has gained credence over the years. However in resource- strapped settings the cost involved in doing AMH levels cannot be ignored. Procuring the result of AMH in smaller cities can also involve long waits. Age, basal FSH and antral follicle count have been suggested as other tests of ovarian reserve which can perform equally well if not better at predicting IVF outcomes 1,2. This study was aimed at investigating the predictive value of Follicle Stimulating Hormone (FSH), Anti-Mullerian hormone (AMH) and Antral follicle count (AFC) on the oocyte yield in IVF cycles. This retrospective cohort study analyses 51 women undergoing IVF in the Assisted Conception unit of a teaching hospital in whom FSH and AMH had both been done on day 2-3 of their menstrual cycle. They also had their antral follicles count (AFC) measured between days 2-3. Antagonist protocol of IVF stimulation was used. Oocyte retrieval was performed under sedation and 2-4 embryos were transferred under ultrasound guidance. Oocyte yield of 4 or less was considered as poor ovarian response and yield above 4 was considered normal response. The primary outcome was the number of oocytes retrieved. Secondary outcomes included grade 1 embryo available for transfer and clinical pregnancy (intrauterine gestational sac on ultrasound scan) rate. The mean age in the study group was 33.2 (SD 4.3) years. The mean FSH was 6.29 (2.28) IU/ml, mean AMH 4.48 (2.87) n/ml and mean AFC was 12.86 (4.93). The mean number of oocytes retrieved were 4.7 (4.4) and grade 1 embryos were 2.5 (1.4). The clinical

pregnancy rate was 29.4% (n=15). There were 11 (21.6%) poor responders and 40 (78.4%) normal responders. The mean number oocytes in poor responders were 3.8 (0.40) as against 10 (4.0) in normal responders. The mean number of grade 1 embryo in poor responders were 2.18 (0.98) and 2.67 (1.49) in normal responders. The clinical pregnancy rate in poor responders was 27.2% (n=3) as against 30% (n=12). The distribution of FSH, AMH and AFC in the 2 groups was analysed using Mann Whitney U test. The median for FSH in poor responders was 6.7 (Interquartile range 4.8, 7.9) and normal responders was 5.8 (5.1, 7.0). The median AMH in poor responders was 4.23 (4.1, 6.1) and in normal responders was 3.86 (2.8, 5.2). The median AFC in poor responders was 11 (8.5, 20.0) as against in 13 (9, 14) normal responders. None of these were statistically significant. However when the oocyte yield was plotted in the IVF failures versus those with clinical pregnancy, interesting trends were observed. FSH below 6 IU/ml and AFC more than 7 correlated well with clinical pregnancies but no such trend could be seen for AMH. Though the FSH, AMH or AFC were not statistically different between the poor and normal responders in terms of oocyte yield, there was trend towards higher oocyte yield with low FSH (< 6.0 IU/ml) and high AFC (> 7). We conclude that these 2 parameters are not only more cost effective but also less time consuming as compared to AMH and hence could be the preferred tests for ovarian reserve in women undergoing IVF in low resource settings.

P4

Recovery of Cyclophosphamide Induced Male Reproductive Toxicities A Nutraceutical Approach

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According to a recent estimate, the incidence of cancer is expected to increase by more than 75% by the year 2030 in developed countries, and over 90% in developing nations. Fortunately, in the past twenty years, because of early detection and improved cancer treatment protocols, the number of men surviving cancer at young age has increased dramatically. Clinically, however, it has been observed that cancer patients receiving chemotherapy experience symptoms resembling andropause. Though in vitro fertility preservation method is available in ART, poor people cannot afford for it. Nutraceuticals could be a promising strategy that can be used in fertility preservation in situ. L-ascorbic acid, α -tocopherol, zinc and selenium besides their physiological roles, including reproductive functioning are also potent antioxidants. The present study was carried out to evaluate the synergistic protective effect of the selected nutrients against testicular toxicities induced by Cyclophosphamide (CP), a broad-spectrum anticancer drug. The experiments were carried out using Swiss albino mice as the test system, which was treated (i.p.) with CP at a dose of 15mg/kg b.wt./week for a period of 5 weeks.

Three doses of the selected nutrients were taken based on their physiological requirements and orally co-administered with the CP. One week post treatment sampling was performed after the final dosing for the seminal analyses, including sperm head abnormality, sperm count, motility and maturation by employing the standard methods. The study was conducted after obtaining the approval from the Institutional Ethical Committee. Results indicate that the selected dose of CP at human therapeutic regimen induces the testicular toxicities in terms of high frequency of abnormal sperms, significant reduction in sperm count and motility. Co-administration of the CP with the selected nutrients resulted in the dose-dependent recovery of toxicities at a significant level. Thus, CP-induced testicular toxicities can effectively be minimized by oral administration of the nutrients. Thus, combination of L-ascorbic acid, α -tocopherol, zinc and selenium can be considered as a nutraceutical package, which imparts the synergistic protective effect against anticancer drugs induced reproductive toxicities in cancer patients.

P5

Role of Protein Supplementation in Modulating the Sperm Genetic Integrity

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Assisted reproductive technology (ART) is a helping hand to those infertile couples who have failed to conceive normally. The success of ART treatment is influenced by several factors, one among the main being the quality of spermatozoa. Various components in the sperm preparation medium are known to enhance the functional competence of the spermatozoa thereby increasing the pregnancy outcome. Studies have shown that protein supplementation in sperm

wash medium induces variety of biochemical changes in sperm membrane however; the effect of varying levels of proteins on sperm DNA integrity has not been elucidated. Hence, the objective of this study was to understand the effect of various concentrations of protein supplementation in sperm wash medium on sperm functional characteristics and genetic integrity. Varying concentrations of Human Serum Albumin (HSA) (0.1%, 0.5%, 1%) were supplemented during

sperm preparation and the processed spermatozoa were evaluated for motility, viability, mitochondrial membrane potential and DNA integrity at different time intervals. Sperm functional competence was significantly increased in 0.5% group in comparison to control

($P < 0.001$). However, DNA integrity was not significantly affected in the processed spermatozoa at various time periods tested. Our results conclude that increasing protein concentration in sperm wash medium has no beneficial effect in protecting the sperm DNA.

P6

Clinical Profile of Ovarian Tumors with Low Malignant Potential: A Case Series

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Among epithelial ovarian tumors around 15% is found to be of low malignant potential. For younger women surgical conservatism is an option. We report a series of women with borderline ovarian tumors. Our aim was to study the clinical profile of women with borderline ovarian tumors. Six women with borderline ovarian tumors diagnosed in the previous two years were studied and the details were compiled and the clinical profile was studied. Of the six women with borderline ovarian tumors four were premenopausal and two postmenopausal. Except one postmenopausal woman all were parous. Abdominal distension (4), pain abdomen (1), abdominal mass (1), postmenopausal bleeding (1) were the presenting symptoms. Four had palpable mass per abdomen, however only one had clinical ascites. Except one all others were clinically diagnosed to be ovarian

tumors- 4 as malignant and one as benign and same was the diagnosis even after ultrasound and/or CT. The CA 125 was above 200U/ml only in one (497U/ ml) who also had fluctuating levels of CA 19-9. Five were mucinous borderline tumors (associated pseudomyxomateronii in one, endometriosis in another) and one was serous borderline variety. One with intestinal type of mucinous borderline tumor was a recurrence 2 years after the primary conservative surgery. The clinical picture as well as imaging studies in borderline ovarian tumors most often simulates malignant ovarian tumors; hence in young women with preoperative diagnosis of ovarian malignancy, utmost caution is necessary while planning the exploratory laparotomy to avoid unnecessary removal of uterus and/or ovaries.

P7

Vitamin C Attenuates Gonadotoxic Effect of Endosulfan in Albino Rats

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Endosulfan is an organochlorine insecticide whose manufacture and usage are being globally banned. Till recently, in India it was used extensively to control pests. It has a low biodegradable property and can enter the human system through water or food chain, mainly due to accumulation of endosulfan in natural stagnant water systems. The aim of the present study was to investigate the protective effect of Vitamin C on endosulfan-induced

gonadotoxic effects in male Wistar rats. Seven day old male Wistar rats were treated with 3, 6, 9 and 12 mg/kg body weight of endosulfan orally from postnatal day continuously for 60 days. In the combination groups, endosulfan (9 and 12 mg/kg body weight), was given after the administration of Vitamin C (20 mg/Kg body weight). Cyclophosphamide (10 mg/kg body weight) was used as positive control. A dose dependent decrease

in body weight, testes weight, sperm count, sperm motility and sperms with abnormal head morphology were observed in endosulfan treated rats. Pre-treatment with Vitamin C resulted in a significant

improvement in these parameters as compared to endosulfan alone. In conclusion, Vitamin C, probably through its antioxidant property reduces the adverse effects of endosulfan.

P8 Effect of Repeated Superovulation on Organelle Function of Oocytes

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Infertility has become one of the leading health concerns in India as well as globally. Ovulation induction and superovulation is commonly practiced in infertility clinics to induce development of multiple follicles in ovaries of women undergoing treatment. However, due to implantation failure or poor ovarian response infertile women undergoing superovulation more than once is quite common. The effect of repeated exposure to supraphysiological hormone conditions on the quality of matured oocytes is still not clear. The present study is conducted to understand the ultrastructural and functional changes in oocytes after repeated superovulation by taking Swiss albino mice as experimental model. Superovulation was induced in mice by injecting 5IU pregnant mare serum gonadotropin (PMSG) followed by 10 IU human chorionic gonadotropin (hCG) after 48h. In multiple

stimulation groups (up to 4 times) a gap of 28 days was maintained between two superovulation cycles. The quality of oocytes obtained was analysed by assessing the cytoplasmic abnormalities and distribution pattern of major cytoplasmic organelles such as of Endoplasmic reticulum, mitochondria and Golgi apparatus. A significant increase in the number of oocytes retrieved increased after superovulation in single as well as multiple doses when compared to unstimulated control. However, there was no significant increase in the oocyte number and distribution of mitochondria and endoplasmic reticulum after repeated superovulation. However the distribution pattern of Golgi apparatus was found to affected, with ~88% of oocytes showing aggregation after fourth dose of superovulation. This study indicates that repeated superovulation does not have any adverse effect on oocyte quality.

P9 Laser Assisted Hatching of Cleavage Stage Embryos Impairs Developmental Potential and Increases DNA Damage in Blastocysts

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This study aims to investigate the influence of two- (day 2) and six-to-eight-cell-stage (day 3) laser assisted hatching on the developmental potential and genetic integrity of the embryos. In this prospective experimental study, two- and six-to-eight-cell-stage mouse embryos were subjected to laser hatching using 1,480 nm diode laser, and then assessed for the developmental potential and DNA integrity in blastocysts. Laser assisted hatching in mouse embryos significantly enhanced the blastocyst hatching potential on day 4.5 ($P < 0.0001$). However, a significant decline in blastocyst total cell number

(TCN) was observed in six-to-eight-cell-stage laser-hatched embryos ($P < 0.001$). Attempt to understand the genetic integrity in laser-hatched mouse blastocysts revealed significantly higher labeling index when hatching was done at two- ($P < 0.01$) and six-to eight-cell stage ($P < 0.05$). DNA damage induced by the laser manipulation may affect implantation and post implantation developmental potential of the embryos. However, further studies are required to elucidate the impact of laser-induced DNA damage on the reproductive outcome.

P10

Effect of Cyclophosphamide on Mouse Epididymis: Histological Analysis

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Chemotherapy is still one of the major treatment modality either as single or in combination with other modalities such as surgery and /or radiotherapy. However, the major concern over its clinical application is its undesirable toxic effects on the normal tissues. Due to the gonadotoxic effect of chemotherapeutic agents the physiological and functional characteristics of gametes have been severely compromised which may lead to infertility. Cyclophosphamide (CP) is an alkylating agent which forms DNA cross linking with its active metabolite. It is one among the widely used cytotoxic alkylating agent with antitumor and immunosuppressant properties. The adverse effect of cyclophosphamide on testicular tissue is well documented. However, its effect on epididymal histology and function is not yet studied in detail. In this direction, the present

investigation is planned to study the histological changes and DNA integrity induced by CP. For the study, male Swiss albino mice were injected intraperitoneally with various doses of CP (50-250 mg/kg, acute dose) and sacrificed at 7, 28 and 42 days after the treatment. The epididymal sections were assessed for routine histological changes by studying the principal and basal cell population. The DNA integrity was assessed by TUNEL assay. The results indicated that CP induced a dose and time dependent changes in the histology of epididymis which recovered after a period of 42 days. Similarly, CP administration resulted in a dose-dependent increase in DNA damage of principal cells. In conclusion CP induces significant histological changes in the epididymis which may have an adverse effect on the sperm maturation process.

P11

Histological Analysis of Human Fetal Testis

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Testicles are the important organ for the production of spermatozoa and testosterone. The present prenatal study of histogenesis of human fetal testis is carried out to know the occurrence of various cell populations like germ cells, Leydig cells, Sertoli cells and peritubularmyoid cells at different gestational periods. The study was conducted on 30 fetus ranging from 10 weeks to 35 weeks. By the end of first trimester of gestation the testicular cords appeared with in the mesenchymal tissue that is from 10- 12th week. By second trimester Leydig cells were appreciated and formed approximately half the volume of total testis. Lobulation with distinct seminiferous tubules was observed. Seminiferous tubule was surrounded by myoid cells and tubules showed presence of 6 to 8 large germ cells. Capsule showed presence of 2 layers. By third trimester differentiation of Sertoli

cell precursors and prespermatogonial cells become well marked that is during 28wk of gestation. Leydig cells were difficult to recognize after 26 weeks. By end of 35 weeks the Leydig cells were in the form of partially regressed juvenile form. All 3 coats of capsule were well demarcated by 28 weeks. Organization of tubules into lobules and seminiferous tubules are prominent between 30 to 34 wks of gestation.

P12

Effect of *Moringa oleifera* Leaf Extract on Busulfan-induced Testicular Toxicity- Histological Changes

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Busulfan is a bifunctional alkylating agent, is used extensively as an antineoplastic agent and as immunosuppressive agent. However, it is reported to have adverse effects on the gonads. Various strategies have been followed in the past to protect the gonadal tissues from drug-induced adverse effects. In the present study we explore the protective effect of *Moringa oleifera* hydroalcoholic leaf extract (MOE) on busulfan (BS) induced testicular toxicity. Swiss albino mice were treated with acute dose of BS (30 mg/kg, intraperitoneally) and/ or multiple doses of MOE (100 mg/kg, 5 days a week for 4 weeks). Mice were sacrificed at 35

days after BS injection and histological changes were assessed in haematoxylin eosin stained paraffin embedded sections. Single dose of BS treatment severely depleted the germ cells from the testicular compartment. Majority of the tubules in cross section did not have complete stages of spermatogenesis and very few tubules had spermatozoa in the lumen. Administration of MOE to BS treated mice significantly increased the germ cells in tubules and few tubules had complete stages of spermatogenesis. The results indicate that MOE has significant beneficial effect on testicular recovery after busulfan treatment.

P13

Catch Me If You Can - A Rare? Aggressive Tumor of the Young

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Villous glandular papillary adenocarcinoma is a sub type of adenocarcinoma of uterine cervix, which is quite rare, recently described, propensity to young women with usually a good prognosis. The frequency of adenocarcinoma of the uterine cervix is increasing and accounts for 10-20% of all cervical cancers. It is also known as Villoglandular adenocarcinoma of the cervix, papillary Villoglandular adenocarcinoma and well-differentiated Villoglandular adenocarcinoma, abbreviated as VGPA. Cervical conization or simple hysterectomy is probably adequate treatment for this subtype. A review of literature showed that only occasional cases showing disease spread, suggesting, contrary to the earlier belief, VGPAs indeed may not be innocent tumours, hence caution must be taken in the management and regular follow up of these patients. Herewith presenting one such case. A 23 year M.Tech student, menarche 13 years, with history of Bronchial Asthma and migraine presented with irregular bleeding P/V for 2 months. Family history was not significant. MRI showed a well-defined

homogenous mass 5.5x5.7 cm from the cervix extending into the endometrium. Tumour markers were normal. P/S cervix unhealthy, polypoid friable mass projecting through the os. P/V and P/R uterus and cervix bulky, no Parametrium involvement. Cervical biopsy was done suggestive of atypical polypoid adenomyofibroma of low malignant potential. She underwent Cervico-isthmic polypectomy. HPR again consistent with atypical polypoid adenomyofibroma of low malignant potential. Cystoscopy was normal. She was asymptomatic for next 4 months, but again had irregular periods, repeat MRI done revealed well defined polypoid minimally enhancing lesion 5.5 x 3cm predominantly central in cervix extending to uterine endometrial cavity. Considering her young age, and low malignant potential with good prognosis, conservative repeat cervicoisthmic polypectomy was done under GA. Histopathological report was Villoglandular adenocarcinoma cervix. Patient and her parents counseled regarding the disease, further management, hysterectomy, with option of ovarian

cryopreservation for future fertility, including surrogacy. Subsequently she had radical hysterectomy with bilateral lymphadenectomy, intraop frozen section of left ovary showed tumor deposits, she had bilateral salpingo-

oophorectomy. Final HPR was Villoglandular adenocarcinoma of cervix, extending to uterus, both ovaries. Lymph nodes were free of tumour. Currently she is on regular follow up.

P14 Effect of Two Different Ovarian Tissue Cryopreservation Methods on the Functional Competence and Genetic Integrity of Oocytes

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Ovarian tissue cryopreservation is the primary treatment modality currently available to women at risk of losing their ovarian function due to chemo or radiotherapy employed in the treatment of cancer. It has been shown that, the oocytes isolated from the ovarian tissue cryopreservation acquire DNA damage during the process of freeze-thawing. Using a mouse model, here we have investigated the functional competence and phosphorylation of H2AX (alpha-H2AX) in germinal vesicle (GV) and parthenogenetically activated oocytes derived from the conventional ovarian tissue slow freezing and vitrification techniques. The number of GV stage oocytes with alpha-H2AX foci was not significantly different between slow freezing and vitrification group. Though, the in vitro maturation (IVM) potential of GV oocytes in slow freezing group showed a significant delay ($P < 0.0001$) in

the process of GVBD, no difference in the maturation rate was observed between the two protocols. Nevertheless, parthenogenetic activation of IVM oocytes using Strontium chloride (SrCl_2) showed significantly lower activation rate in slow freezing group compared to vitrification ($P < 0.05$) and control ($P < 0.01$). Importantly, reduced H2AX phosphorylation was observed in slow freezing group in comparison to control ($P < 0.05$). Therefore, we conclude that the sensing of DNA strand breaks and impaired repair process are associated with the functional competence of the oocytes recovered from slow freezing group which may have a significant impact on the reproductive outcome. Hence vitrification may be preferred over slow freezing for the cryopreservation of ovarian tissues in oncofertility programs.

P15 Umbilical Vein Blood Volume Flow Rate and Umbilical Artery Pulsatility as 'Venous -arterial Index' Predicts Adverse Perinatal Outcome

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Quantification of umbilical vein blood flow rate might be valuable in assessing fetuses at increased risk of perinatal complications as they receive their supply of oxygen and nutrients via this vessel. As it is a single vessel, blood flow could be measured at any site along its length. The study objective was to compare umbilical venous arterial index (VAI) with adverse perinatal outcome and also to evaluate its efficacy with other flow indices in determining perinatal outcome. Various Doppler indices such as normalized blood flow rate in umbilical vein (nUV, ml/kg estimated fetal weight/min), venous-arterial

index (VAI; nUV/UAPI), umbilical artery resistance index (RI), umbilical artery pulsatility index (UAPI) and S/D ratio were determined in 103 pregnant women within 2 weeks of the delivery. A risk score was devised using APGAR at 5 min, birth weight, preterm delivery, fetal distress, NICU care and perinatal death and this score was correlated with antenatal Doppler findings. Subjects with low VAI were found to have a greater association with IUGR fetuses (28.5%) and low liquor (35.7%), preterm deliveries (46.4%), lower mean birth weight (2.25kg), higher NICU admission rates (32.1%).

Unfavorable score was noticed in 25.2% of the neonates. They had lower VAI (156 vs. 241), UVD (6.2 vs. 7.8), UVel (16.2 vs. 17.8), nUV (163.7 vs 206.4) and higher PI (1.3 vs. 0.9). A cut off of VAI of 105 ml/kg/min had sensitivity of 86.7% and a specificity of 93.5% for predicting poor perinatal

outcome. It was concluded that VAI with a cut off of 105 ml/kg/min can be used as an additional investigation tool alongside the other conventional diagnostic tools used in current Obstetric practice in order to predict adverse fetal outcome.

P16

Effect of Curcumin and its Novel Derivative on Oxidative Stress Generated During in vitro Maturation of Oocytes

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In vitro maturation (IVM) of oocytes has gained lot of significance in assisted reproductive technology and fertility preservation field. Even though it has several advantages, the oocytes matured under in vitro conditions generally exhibit poor cytoplasmic organization and functional competence. It is increasingly evident that these changes are partly driven by oxidative stress generated during in vitro culture conditions. In this context, supplementation of culture medium with free radical scavengers seems to be an attractive option. Curcumin is a polyphenol with potent antioxidant property. However, its poor solubility limits its application. Therefore, in the present study we assessed the beneficial effect of

curcumin and its novel derivative on oxidative stress during IVM. The oocytes were cultured in F12 medium supplemented with various doses of curcumin or its novel derivative. At 24h later, the nuclear maturation and intracellular ROS (DCHFDA method) level was estimated. Both curcumin and its novel derivative exhibited beneficial effect on the nuclear maturation of oocytes. In addition, a lower intracellular ROS level was observed in these oocytes. The results indicate that curcumin and its novel derivative may offer significant beneficial effect on the developmental competence of oocytes by reducing the oxidative stress generated during in vitro culture.

P17

Effect of Ejaculatory Abstinence on Human Semen Quality and Sperm Functional Characteristics

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Mature spermatozoa released from the seminiferous tubules of the testis undergo final maturation in the epididymis. Though epididymal storage of sperm is important to acquire its fertilizing ability, prolonged storage may affect its genetic and functional ability. WHO recommends 2-7 days ejaculatory abstinence prior to any therapeutic intervention. So far the influences of ejaculatory abstinence on specific sperm characteristics are not elucidated. Hence, the objective of this study is to study the sperm characteristics in relation to the ejaculatory

abstinence. In this retrospective study, data from 2000 subjects attending university fertility clinic for routine semen analysis were included. Semen characteristics such as volume, sperm concentration, motility, morphology and viability were assessed in relation to different abstinence periods. Though there was no significant difference seen in morphological characteristics, sperm viability and motility was lower in day 7 ejaculatory abstinence. The data related to specific sperm characteristics and ejaculatory abstinence is being analysed and will be presented.

P18

Effect of Metformin on Pre-implantation Embryo Development

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Polycystic Ovarian Syndrome (PCOS) is one of the leading causes of anovulatory infertility in females, especially in adolescents. Metformin, a glucophagic drug which acts by sensitizing cells towards insulin for glucose uptake, is commonly prescribed to PCOS patients as they are prone to develop insulin insensitivity leading to diabetes mellitus type II. It is also prescribed to such patients during pregnancy to avoid gestational diabetes mellitus. However, its effect on early embryo development is not clearly known. Therefore, the present investigation was planned to study the effect of metformin on pre-implantation embryo development. Adult female Swiss albino mice were superovulated and kept for mating with

healthy male mice. Embryos at 2 cell stage were obtained and cultured in vitro in KSOM media supplemented with various concentrations (0, 10, 25, 50, 100 µg/ml) of metformin. The developmental potential, blastocyst rate and DNA integrity were assessed. Metformin exposure did not show any effect on embryo development until morula stage whereas post compaction stage a decline in developmental potential was seen in the highest dose (100 µg/ml). However, a dose-dependent decrease in the DNA damage with increasing metformin concentration was observed in blastocysts. The results suggest that metformin does not have any adverse effect on pre-implantation embryo development at lower doses.

P19

DNA Integrity in Oocytes and Granulosa Cells Subjected to Ovarian Tissue Cryopreservation by Slow Freezing and Vitrification

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An unfortunate consequence of the cytotoxic treatments currently used in cancer treatment is infertility. Increasing incidence of cancer survival has led to an urge to preserve the future reproductive potential of patients. Cryopreservation of ovarian tissue has emerged as a promising option for such patients. Conventional slow freezing using a programmed freezer and vitrification by direct plunging into liquid nitrogen are common methods used for ovarian tissue cryopreservation. The freeze thaw process can substantially increase the risk of oocyte damage, which may eventually compromise its quality and consequently have adverse effects on the embryo and reproductive outcome. Thus the present study was designed to evaluate the effect of mouse ovarian tissue cryopreservation by vitrification and standard controlled slow freezing techniques on oocyte and granulosa cells DNA integrity. The DNA integrity in

oocytes and granulosa cells was measured by the single cell gel electrophoresis which demonstrated an increase in oocyte and granulosa cell DNA damage in both vitrification and slow freezing. However the oocytes retrieved from the vitrification group had significantly lower DNA damage than that of slow freezing. Further, oocyte mitochondrial integrity was superior in the vitrification group. Thus it can be concluded that ovarian tissue subjected to vitrification is more tolerant to cryoinjury and therefore appears to be a promising alternative for cryopreservation of ovarian tissues.

P20

Methyl Parathion Inhibits the Nuclear Maturation, Decreases the Cytoplasmic Quality in Oocytes

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Methyl parathion is an organophosphorus and extremely hazardous pesticide, it is a most commonly used pesticide and acaricide in India. Even though a large number of the studies indicate the testicular toxicity, not much information is available on female reproductive system. In this study we report the adverse effects of methyl parathion on the female reproductive system using Swiss albino mice as the experimental model. MP was administered orally with 5, 10 and 20 mg/kg of body weight. One week after the treatment mice were superovulated with pregnant mare serum gonadotropin (PMSG) and human chorionic

gonadotropin (hCG) to study the quality of the oocytes and spindle organization. MP exposure resulted significant inhibitory effect on the nuclear maturity of oocytes, which was associated with spindle deformity and higher cytoplasmic abnormalities with depleted glutathione level. In addition, the number of primordial follicles significantly decreases following MP exposure. The result of the study clearly indicate that MP has adverse effect on the oocyte which may have impact on the fertility status as well as the pre- and post natal development in the offspring.

P21

Moringa oleifera Leaf Extract Mitigates the Cyclophosphamide Induced Apoptosis in Testicular Germ Cells

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Moringa oleifera L. commonly known as drumstick tree, is widely cultivated in India due to its nutritional and medicinal significance. The present investigation is planned to explore possible chemoprotective effect of *Moringa oleifera* leaf extract (MOE) against cyclophosphamide (CP) induced testicular germ cell toxicity. Male Swiss albino mice (2 week) were intraperitoneally injected with acute dose of phosphate buffered saline (Control), CP (200 mg/kg body weight) and MOE (100 mg/kg body weight). In combination group, MOE was administered 24h prior to cyclophosphamide (MOE+CP). One week later, testicular germ cells were isolated for DNA integrity assessment by comet assay and m-RNA expression of apoptotic regulators (P53, BAX, BCL2, Cytochrome C, FAS, FASL and C-kit) by quantitative real time PCR. Significant increase in

DNA damage ($p < 0.05$) clearly indicated the germ cell toxicity of CP. Further there was increase in expression of apoptotic genes P53, BAX, Cytochrome C, FAS, FASL and decrease in anti-apoptotic gene BCL2. C-kit, a key factor which determines the survival of stem cells, was also significantly down-regulated ($p < 0.05$) after CP treatment. Co-administration of MOE counteracted the CP-induced germ cell toxicity by down-regulating apoptotic genes, up-regulating anti-apoptotic genes and C-kit. In conclusion, chemoprotective property of MOE is mediated through altering the expression of genes involved in apoptotic regulation and stem cell survival.

P22

Effect of Isoniazid Treatment on Testicular Function in Swiss Albino Mice

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Tuberculosis (TB) is the most common infectious disease and is the second leading cause of death due to a single infectious agent. Isoniazid is one of the first line anti-tuberculosis drugs. It interferes with synthesis of nucleic acids and phospholipids as well as elongation of fatty acids. Isoniazid is known to cause side effects including hepatitis, hypersensitivity and peripheral neuritis. However, the effect of isoniazid on the gonadal function is not reported yet. Therefore, the present study was aimed at studying the effect of isoniazid on testicular function. Adult male Swiss albino mice were treated with varying doses of isoniazid (19, 39 and 78mg/kg body weight) intraperitoneally, 3 times a week for 4 weeks. Caudal sperm were

collected at 2 weeks after the completion of the treatment. The mice injected with the lowest dose showed significant increase in sperm output ($p < 0.05$) when compared to control, whereas in the higher doses the increase was not significant. Isoniazid treatment did not have any significant effect on sperm parameters such as sperm count, motility and chromatin immaturity, however, a non-significant increase in head defects was observed in the mice treated with highest dose of isoniazid. The results of the study indicated that isoniazid may not induce any significant adverse effect on male testicular function.

P23

Protective Effect of Indian Propolis against Mitomycin C induced Gonadotoxicity

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Chemotherapy is one of the widely used treatment modality in cancer cure. However, its gonadotoxic effect is a serious concern in cancer survivors. Various approaches have been attempted in the past to prevent the gonadal toxicity induced by chemotherapeutic drugs. Combination therapy with biological agents such as vitamins, antioxidants, dietary supplements and plant products are considered as an attractive option. Indian propolis is a complex resinous material with potential medicinal properties including antibacterial, anti-inflammatory and anti-oxidative properties. In the present study we investigated the protective role of propolis on gonadotoxicity induced by chemotherapeutic drug mitomycin C (MMC). Healthy adult male mice were divided into four groups and injected intraperitoneally with saline, MMC (8mg/kg body weight, single dose) and Propolis (400mg/kg body weight, 5days a week for 4 weeks). In combination group mice were injected with MMC (8 mg/kg, single dose) and

Propolis (5days a week for 4 weeks). The animals were dissected at 35 days after MMC treatment to assess epididymal parameters and oxidative stress in testis. MMC administration significantly reduced the testicular weight, sperm concentration and motility. In addition, there was significant increase morphologically abnormal and DNA damaged spermatozoa in MMC treated mice. Propolis administration prevented loss of testicular function by protecting it from the MMC induced oxidative stress. The present study provides evidence that pretreatment with propolis effectively decreases MMC induced gonadal toxicity and therefore may be considered as an adjuvant therapy as a mode of fertility preservation.

P24

Antiretroviral Drugs: a Comparative Analysis

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Antiretroviral therapy has led to a sustained decline in HIV-associated mortality and life expectancy is now approaching general population by HAART. But, efficacy of antiretrovirals is compromised by toxicity, resistance and incomplete adherence. Therefore antiretroviral use is not limited to treatment of chronic infection. Thus the objective of this study is to compare newly emerging antiretroviral agents. Indole-containing compounds with potential to inhibit HIV-1 fusion by targeting hydrophobic pocket of transmembrane gp41 and Peptides have been developed into drug candidates useful in salvage therapy against HIV strains resistant to HAART. Nevirapine, or Efavirenz lead to rapid development of drug resistance. Etravirine and Rilpivirine were approved to manage treatment-experienced HIV-1 infected people, and

naïve and adult patients respectively. Despite that, viral resistance associated to NNRTI drugs and adverse effects continue to emerge in chronic long-term treatments. So, there is pressing need for new antiretroviral agents. Introduction of two methyl groups at positions 30n and 50 of the 3-phenylsulfonfyl moiety led to IAS derivatives having broader spectrum of activity against mutant HIV-1 strains. Space surrounding the 2-carboxamide proved to tolerate a wide variety of substituents; enhancing potency of IAS analogues. Also tolerability, pharmacokinetic profile and efficacy of Isentress were evaluated in HIV-1 infected children and adolescents 2 to 18 years of age (open-label, multicenter clinical trial, IMPAACT P1066). The safety profile was comparable to that in adults.

P25

Biotin Enhances Human Sperm Motility and Developmental Potential of Mouse Embryos

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Motility is a characteristic function of the male gamete, which is required for the sperm to reach the oocyte and fertilize. Reduced number of progressively motile sperm limits the success of assisted reproductive techniques (ART). Therefore, sperm motility enhancers have immense clinical significance in ART. Biotin, a water soluble B-complex vitamin, is essential for all carboxylation reactions. Biotin non-significantly enhanced the motility compared to control. The

motility enhancement was more pronounced in asthenozoospermic samples. In both normozoospermic and asthenozoospermic samples the increase in motility and longevity of spermatozoa was similar to pentoxifylline. Further, biotin enhanced developmental potential of 2 cell stage embryos when cultured in vitro. The results clearly indicate that the biotin can be used as a safe sperm function enhancer.

P26

Protective Effects of Piper Betle and Piper Nigrum Against Haematological and Histological Damage Induced by High Energy e-Beam Radiation

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In the present investigation, in vivo radioprotective effect of Piper betle and Piper nigrum extracts against high energy e- beam radiation was studied using haematological and histological parameters

in Swiss albino mice. For all haematological and histological studies a radiation dose of 8 Gy was selected. Three doses of the plant extracts, viz., 20, 40 and 80 mg/kg.b.wt. were chosen for

haematological studies and for histological studies 80 mg/kg.b.wt. was chosen. The animals were pretreated with the extracts prepared in the solvent, carboxymethyl cellulose (CMC) through oral gavage for seven consecutive days and one hour after the last treatment, they were subjected to whole body irradiation. Haematological studies were carried out at five different time intervals, viz, 24hr, 48hr, 72hr, 7 days and 14 days after irradiation and the histological preparation of small intestine was done after 3 days of irradiation. There was a steady decline in the TEC, TLC and Hb content on the 3rd day. There was a significant increase in the total erythrocyte count (TEC), total leucocyte count (TLC) and hemoglobin (Hb) content in radiation exposed animals treated with the plant extracts in a dose- dependent manner. Restoration was seen after the 7th day and recovery rate was slower during the earlier days

after radiation exposure. The rate of restoration of Hb content was at a slower pace when compared to RBC and WBC counts. In the irradiated group, the villus height and mucosal length of the small intestine were found to be significantly shorter compared to the sham control, demonstrating the damaging effects of irradiation on the intestinal mucosa. It was observed that irradiation decreased the number of surviving crypts significantly and caused mucosal thinning. Both *P.betle* and *P.nigrum* extracts showed histoprotective effects; the former being more potent than the latter. The extract treated groups showed a significant increase in the number of surviving crypts in the small intestine compared to radiation alone group. The villus height, crypt depth and mucosal length in the combined treatment group were found to be greater than those of the radiation control group.

P27

Morin Enhances Motility of Frozen-thawed Spermatozoa

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Cryopreservation of human gametes has become an integral part of assisted reproductive technology (ART). Decrease in motility, mitochondrial damage and loss of DNA integrity are the most common consequences of freeze-thaw process which is thought to be mediated through reactive oxygen species. Under such circumstances pharmacological agents like pentoxifylline, caffeine etc are generally used to enhance sperm motility. However, their reported toxicity on gametes and embryos limits their clinical application. Morin (3,5,7,2',4'-pentahydroxyflavone), a natural flavonoid is the primary bioactive component of the family Moraceae. Since morin is a potent antioxidant, the present investigation was aimed at exploring its role in enhancing the sperm function under in-vitro conditions. Frozen-thawed semen samples from the infertile men were incubated in medium with or without morin (5µg/ml). Sperm motility, mitochondrial integrity and kinematics were assessed. Motility of spermatozoa was enhanced by 10-12% with supplementation of morin to

sperm wash medium. Similarly results of rhodamine 123 assay revealed a higher percentage of spermatozoa with intact mitochondria in morin supplemented group when compared to control. The results suggest that morin has beneficial effect on frozen-thawed sperm function under in-vitro conditions.

P28

Effect of Prepubertal Testicular Tissue Vitrification on Apoptotic Response

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With the increase in survival rate of prepubertal cancer patients, need for their fertility preservation is emerging as the agents used in cancer treatment have profound adverse effect on germ cells. Cryopreservation of testicular tissue is considered as the only best option in prepubertal cancer affected boys for their fertility preservation. Prepubertal testicular tissue cryopreservation is still experimental and impact of freeze thawing process in relation to the age of the tissue on apoptotic response has not been elucidated so far. Hence the objective of this study was to elucidate the effect of prepubertal testicular tissue vitrification on viability, DNA integrity and apoptotic response using a mouse model. Testicular tissue from one to four week old mice were collected and subjected to vitrification. After thawing the tissue,

viability was assessed. Expression of DNA damage response gene p53 and apoptotic genes viz. bax, bcl2 levels were assessed by real time PCR. In addition, flow cytometric assessment was performed to quantify the extent of DNA damage in testicular tissues. Cell viability was significantly reduced by freeze thaw process in comparison to fresh tissue. However, no significant variation in the viability was observed in the post thaw cells between different age groups. In contrast, difference in the expression of apoptotic genes was observed in relation to the prepubertal age. These observations suggest that the apoptotic response to freeze-thaw process vary in relation to the tissues. This information will have a potential implication in oncofertility program.

P28

Effect of Zinc oxide Nanoparticles on Human Spermatozoa

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Advances in nanotechnology engineering in recent decades have given rise to rapid development of many novel applications for nanoparticles (NP) in various fields. Zinc oxide (ZnO) NP which have their own importance due to their vast area of applications in creams, paints, manufacture of concrete, food additive etc., act as Inorganic UV filters and has been used as ingredients in sunscreens for over three decades. Due to their high penetration ability, they can cross biological barriers such as blood-brain, blood-testis and placenta. Since testis has high proliferating germ cells, ZnO NP may have effect on sperm function. Therefore in the present study we intended to investigate the effect of ZnO NP on human sperm function. Ejaculates from men attending Andrology laboratory, Kasturba Medical College, Manipal were used in this study. Motile spermatozoa were

extracted and incubated with different concentration of ZnO NP (0, 10, 50, 100, 200 µg/ml) up to 24 hrs. at 37°C and 5% CO₂. The motility of spermatozoa decreased with the increased dose of ZnO NP. However, it prevented the loss of DNA integrity of spermatozoa during in vitro incubation. Thus the results suggest that though ZnO NP has inhibitory effect on motility of human spermatozoa it preserves DNA integrity during in vitro incubation.

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