ASSESSMENT OF EFFICACY OF PIPPALYADI YOGA ON NORETHISTERONE INDUCED ANOVULATION IN ALBINO RATS

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Submitted By

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Undertaking

I Dr **Gayathri Bhat.N V** is the Ph. D Scholar of the Tilak Maharashtra Vidyapeeth in Prasuti Tantra and Stree roga . Thesis entitled **"Assessment of Efficacy of Pippalyadi Yoga on Norethisterone Induced Anovulation in Albino Rats"**under the supervision of Dr. Praphullata Prashant Suru, solemnly affirm that the thesis submitted by me is my own work. I have not copied it from any source. I have gone through extensive review of literature of the related published / unpublished research works and the use of such references made has been acknowledged in my thesis. The title and the content of research is original. I understand that, in case of any complaint especially plagiarism, regarding my Ph.D. research from any party, I have to go through the enquiry procedure as decided by the Vidyapeeth at any point of time. I understand that, if my Ph.D. thesis (or part of it) is found duplicate at any point of time, my research degree will be withdrawn and, in such circumstances, I will be solely responsible and liable for any consequences arises thereby. I will not hold the TMV, Pune responsible and liable in any case. I have signed the above undertaking after reading carefully and knowing all the aspects therein.

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Annexure IV

CERTIFICATE BY THE SUPERVISOR

It is certified that work entitled **"Assessment of Efficacy of Pippalyadi Yoga on Norethisterone Induced Anovulation in Albino Rats"**is an original research work done by Dr.Gayathri Bhat.N.V under my supervision for the degree of doctor of philosophy in Prasuti Tantra and Stree roga to be awarded by Tilak Maharashtra Vidyapeetha, Pune.

To best of my knowledge this thesis

- Embolies the work of candidate herself
- Has duly been completed
- Fulfils the requirements of the ordinance related to Ph.D. degree of the TMV
- Up to standard in respect of both content and language for being referred to the examiner.

Signature of Supervisor

Dr. PraphullataPrashant Suru

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Dr. Gayathri Bhat N V

LIST OF ABBREVIATIONS USED

A.H.	Astanga Hridaya
A.S.	Astanga Samhita
BN	Bhavaprakasha Nighantu
B.P.	Bhava Prakash
C.D.	Chakradatta
Cha.	Charaka Samhita
DN	Danvantari Nighantu
G.N.	Gada Nigraha
Hb	Haemoglobin
KN	Kaiyadeva Nighantu
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
M.N.	Madhava Nidana
MN	Madanapala Nighantu
PN	Priya Nighantu
PCV	Packed Cell Volume

RN	Raj Nighantu
RDW-CV	Red Blood Cell Distribution Width Coefficient Variance
RDW-SD	Red Blood Cell Distribution Width Standard Deviation
Sha.Sa	Sharangadhara Samhita
Sho.N	Shodala nigahantu
TC	Total Count
WBC	White Blood Cells

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ABSTRACT

Reproduction is one of the prime characteristics of living creatures to continue its species. In this society having a child is a reputation as by the child the parents get liberated by all bonds. When all factors responsible for the fertilization are healthy and free from abnormalities then the result will be in time conception and birth of healthy progeny. Vandyatwa is inability of the female to reproduce and is the result of Artava dusti and Yoni dosha. Nastartava is the condition where there will be anovulation leaving the woman in barren state. Increasing incidence of infertility world over has necessitated the search for newer management protocols to treat infertility successfully. As the modern scientific world demands for evidence based practices it is a need of the hour to do research in already classically documented treatment modalities. As oestrous cycle of Albino rat is short it was selected for the experimental study.

Pippalyadi yoga which contains Trikatu and Nagakeshara mentioned in Bhaishajya Ratnavali for the treatment of vandyatwa was selected for experimental study.

Aim: To evaluate the effect of Pippalyadi yoga on induction of ovulation.

Objectives

1. Phytochemical Analysis 2. Acute toxicity study 3. Efficacy study

Study design: 36 Albino rats were grouped into 6 groups. Test drug Pippalyadi yoga was administered for 21 days including experiment day in the morning session between 9-10am orally after taking the cervical smear.

Change of drug:

Due to Market Ban of Di-ethylstilbestertol , NORETHISTERONE was taken as the control drug and the certificate of change of drug was taken from the research center SDM, UDUPI. In case of 5^{th} group and 6^{th} group first NORETHISTERONE was administered subcutaneously between 9-10 am after taking the cervical smears then after 1 hour Pippalyadi yoga was administered orally.

This experimental study shows positive changes in the estrus cycle with test drug Pippalyadi yoga. The drugs Trikatu and Nagakeshara show estrogenic activity hence increased thickness of endometrium was observed. This also suggests that the drug promotes the follliculogenesis.

The toxicity study shows no adverse effect on the animal.

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CHAPTER 1
INTRODCTION

तत्र द्वयोर्दम्प्त्यो:स्वभावत् स्वकर्मपरिणामाद्वाप्रजाभिर्व्रुधिर्भवति ।
तौ धन्यौ, अतो अन्यथा भिषजितव्यौ॥ Ka.Sha.5/3

Kashyapa says couple who can reproduce within one year naturally are considered to be blessed otherwise they should be treated. Infertility is a condition which can be defined as inability to conceive with minimum one year of unprotected sexual life. Global estimate for infertility in women between the age of 20 to 44 ranges from 1.5% for primary infertility and 3% for secondary infertility with chronic anovulation in about 6- 15%[1]. Inability of couples to fulfil their desire of continuing the family has been a social stigma since ages. There may be good number of reasons for this condition to occur. Increasing awareness on the treatment of infertility has made it easy to seek help to resolve the problems with conception.

The causes for this condition are due to problems in health of female and male reproductive system, in its structure and physiological function, genetics and immunological factors. Psychology of the couple, environmental factors and sometimes unexplained causes also may account for this condition. Successful conception demands sequential process like timely ovulation, ovum pick up, sperm deposition, sperm motility, fertilization, transportation of embryo to the uterine cavity by healthy fallopian tube. Vandyatwa is a condition where a female fails to conceive or unable to sustain pregnancy or give birth to live baby or is unable to conceive after previous pregnancy. For Vandyatwa there are several etiological factors that are mentioned in Ayurveda classics like, Yonivyapath, Artavadushti, Manasikadosha, Shukradosha, Daiva. The factors like Mithyaahara, vihara, Manasikadoshas hamper basic conception factors like Ritu, Kshetra, Ambu, Beeja and will lead to infertility[2]. Hence the integrity of these four factors is important for the achievement of conception to get Shreyasi Praja. Even the Shadbhavas such as Matrija (maternal), Pitraja(paternal), Atmaja (soul),

Rasaja(nutritional), Satmyaja (wholesomeness) and Satvaja (psych/mind) play an important role in conception of a healthy progeny with highest quality. Therefore, a healthy mother and father practices of a wholesome regimen and a healthy mind plays a prime role in achieving a healthy offspring.

As there are many causes for Vandyatwa mentioned in classic Samhitas, Vandyatwa due to Artava Nasha or Nastartava where Artava is taken as ovum was taken for the study. There are number of ovulation induction drugs are in use but they are reported to have various adverse effects, such as ovarian enlargement, vasomotor flashes, nausea, vomiting, breast discomfort, headache, abnormal vaginal bleeding, visual symptoms, weight gain and shortness of breath. Clomiphene citrate the drug commonly used to induce ovulation is reported to have many side effects like acute pancreatitis, deep venous thrombosis, myocardial infraction, endometrial carcinoma etc. Hence there is a need to find some safe and effective drug for the induction of ovulation.

Many yogas are mentioned by our Acharya's, Pipplayadi Yoga which contains Trikatu and Nagakeshara is one among them mentioned in Bhaishajya Ratnavali Yoniroga Chikitsa for Vandyatwa.

Rats are selected for many research related to reproduction as they have short estrous cycle. In rats many medicinal plants have shown fertility effects. The morphological changes happening in the uterus, ovary and vagina determine the estrous cycle. The different phases of estrous cycle are identified by the types of cells present in the vaginal smear.

Pippalyadi yoga with Pippali, Maricha, Shunti and Nagakeshara is having Deepana Pachana properties and helpful in the Amapachana there by resulting in the production of healthy unvitiated upadhatu Artava by Rasa Dhatu and maintain the normal functioning of Artavavaha Srotas. Hence Vandya will conceive.

Thus, in the present study the fertility effect of Pippalyadi yoga was studied on Norethisterone induced anovulation in female albino rats taking different parameters.

CHAPTER 2

AIMS AND OBJECTIVES

1. AIMS

To evaluate the effect of Pippalyadi yoga on Norethisterone induced anovulation in Albino Rats

2. OBJECTIVES

1. Phytochemical Analysis of Pippalyadi Yoga
2. Acute toxicity study of Pippalyadi Yoga
3. Efficacy study of Pippalyadi Yoga

CHAPTER 3

RESEARCH QUESTION AND HYPOTHESIS

RESEARCH QUESTION –

Is Pippalyadi Yoga effective in the management of Norethisterone induced anovulation in Albino Rats?

HYPOTHESIS –

1) Research hypothesis –Pippalyadi Yoga is effective in Norethisterone induced anovulation in Albino Rats.

2) Null hypothesis – Pippalyadi Yoga is not effective in Norethisterone induced anovulation in Albino Rats.

CHAPTER 4
PREVIOUS WORK DONE

1. Role of Prajasthapana gana siddha Ghrita Uttarabasti in the management of Vandhyatwa W.S.R to Beejotsarga was done by Swati S Jadhav. IPGT & RI Gujarat Ayurveda University Jamnagar in 2002. In this study, considering overall effect of Uttarabasti and oral Prayoga, 29% people had ovulation while all the patients have shown increase in follicular size.

2. Pharmacognostical and phyto-chemical evaluation of Pippalyadi yoga a polyherbal formulation Pandya Shachi Hemantkumar1, Kamini Dhiman, L. P. Dei, A. B. Thakar, Harisha C R.

3. A clinical study on the efficacy of Ashwangandha Ksheerapaka in Streevandhyanthva wsr to anovulation by Rajani Kagga from RGUHS, Karnataka, Bangalore in 2016. The result stated that out of 30 patients maximum 93.3% there was no change observed in the follicles where as in 6.7% there is slight improvement in follicular growth also the endometrial thickness, but this is not statistically significant.

4. a clinical study on effectiveness of Puga Paka in the management of Stree Vandyatva dur to anovulation by Dr. Shruthi from RGUHS, Karnataka, Bangalore in 2017. The result stated that out of 26 patients maximum 47.61% there was no change observed in the growth of follicles where as in 33.3% there is slite improvement in follicular growth also endometrial thickness, 19.04% got ovulation. It is statistically significant.

5. To study the efficacy of Narayan Taila Uttarabasti in female infertility with special reference to anovulation was done Monika Chauhan from RGUHS ,Karnataka and Bangalore in 2016.The result stated that out of 30patients ,3.33% of patient i.e one patient conceived. Complete remission i.e ovulation was found in 30% of patients .While 56.67% of patients were reported with improvement and 16.675 , Patient had no response to the treatment.

CHAPTER 5

LITERARY REVIEW

5.1 Ayurvedic review

In this chapter the references about the Nastarthava, Vandhyatwaand chikitsa has been taken from different Ayurveda Samhitas.

Nirukti –

Vandhya - "यस्यगर्भधारणमागभरुपंबन्दनंसंप्यतिसावन्द्या।"श.कद्रु.

In woman whom the Garbhadhaarana marga is blocked she is called as Vandya

Paibhasha

वन्द्यानस्टार्तवविद्यात्॥su.u.38/10

The female in whom Artava is lost is called Vandya

Vyutpati

The word Vandhya is derived from the root "Vandh" + "Yak", which meaning sterile, Un-fruitful waste.

Vandhyatva is a condition where the female is unable to conceive even after unprotected intercourse.

Synonyms

वसा:-वशावन्द्यवेति॥[3]

अवतोक :- अवपततितोकंअपथ्यंवागर्भोवाअस्यांइतिअवतोक॥[4]

अवकेशि :- अवच्युतंकंसुखंपुत्रसुखंप्रसवसुखंवातरिशांतुशीतमात्राइतिअवकेशि॥[5]

स्रवतागर्भा:- स्रवगर्भायस्या: सास्रवतागर्भा॥[6]

अफला:- फलंनास्तियस्यासाअफला॥[7]

Classification

The Classification of Vandhyatva has been mentioned in Harita Samhita, Rasa Ratna Sammuchaya and Vandhya Kalpadruma.

Acharya Charaka[8] **-** has mentioned

1. In the etiology the word-Sapraja
2. In the clinical features of Asrujayonivyapath the word – Apraja[9]
3. Under congenital abnormalities the word-Vandhya

Harita Samhita: The classifications of Vandhya are as follows- [10]

1. Kakavandhya: women are unable to conceive again who has child from previous

conception.

2. Anapatya: - The woman who has never conceived.
3. Garbhasravi: - The woman with repeated abortions.(before third month)
4. Mritvatsa: -The women who has conceived but unable to deliver live child.
5. Balakshaya: - Infertility in malnourished women who doesn't have enough strength to sustain pregnancy (strength or auto immune disorder)
6. Balya/Dhatukshayaja:- Infertility due to balayaavastha or dhatukshayaavastha

Rasa Ratna Samuchchaya

Nine types of Vandhya have been described.[11] they are- Adivandhya, Vataja, Pittaja, Kaphaja, Sannipataja, Bhutaja, Daivaja, Raktaja, Abhicharaja.

V**andhya Kalpadruma**

Eight types of Vandhya are described according to the causes.[12]

1. Tripakshi
2. Subhrati
3. Sajja
4. Trimukhi
5. Vyaghrini
6. Baki
7. Kamali
8. Vyaktini

Vandhyatva Nidana

Before discussing the Nidanas of infertility, need to know the essential factors for conception. Abnormalities in these factors may lead to Vandhyatva. [13]

ध्रुवं चतुर्णां सानिध्यात् गर्भ स्याद् विधिपूर्वक:।

ऋतुक्षेत्राम्बुबीजानांसामाग्र्याद्यादंकुरो यथा ॥ सु. शा. 2/33

Acharya Sushruta has mentioned this under the heading of Garbhasambhavasamagri.

Ritu, Khestra, Ambu, Beeja are considered as the four essential factors forthe conception. Along with these Vaghbhatacharya adds unvitiated Anila and hridaya as the essential factors for the birth of progeny with all desired qualities.

शुद्धेगर्भाशयेमार्गेरक्तेअनिलेहृदिवीर्यवन्तंसुतंसूते॥A.H. Sha.1/7

Rutu

Here Rutu means fertile period during ovulatory cycle. Conception is tried during this

period only, for the achievement of the conception.

ऋतुअन्गनायरज: समय: । सु श.दल्हण्टीका

Here Rutu means fertile period during ovulatory cycle. Conception is tried during this period only, for the achievement of the conception.

ऋतुश्चनिषिक्तस्यबीजस्यफलप्रसवानुगुणकाल: | A.S.Sh.1/10 indu)

During this period the pumbeeja which has entered the yoni will form the phala (garbha) hence this period is Anukula for the conception.

ऋतुमतिलक्षण-

पिनप्रसन्नवदनप्रक्लिनात्मामुखाद्विजाम्नरकामाम्प्रियकथांस्रस्तकुक्ष्यक्षिमूर्धजाम्॥

स्फुरद्भुजकुचश्रोणिनाभ्यूरुजघनस्फिचाम्।हर्षोत्सुक्यपराम्चापिविद्याद्रुतुमतिंइति.॥Su.sha.3/7-8

During this period the garbhashaya is free from puraana rajas and is shudha and beejarupiArtava is also being formed. Means it is the state of wellbeing in relation to female reproductive system while it is nourished by Shudha Artava. Artava is agneya and after the formation as a Upadhatu of rasa it circulates through- out the body by the action of unvitiated VyanaVata and exhibits the laxanas of Rasa sarata and indicates the prakrutavastha of Agni.

All these features are due to the influence of estrogen. It improves the mood, energy level and general wellbeing.

Ritu also can be taken as **age of the couple** as well as the **time of coitus** in a broader sense. And time duration is again classified as Adana and Visarga

Vaya (Age) of couple

पन्चविंशेततोवर्षेपुमान्नरितुषोडशे।समगतवागतवीर्यौतौजानीयात्कुशलोभिषक्॥Su.Su.35/13

As for the male by the age of 25 and for female at the age of 16 there will beDhatuparipurnata and they attain mental and physical maturity, they should try for conception to get a child with all possible good qualities.Before this age if conception is tried then it may be fruitless or the fetus may get abnormalities of different magnitude and also the man will be diseased due to improper and untimely use of hisstrength.

Kaala (Time)

By the influence of Surya and Chandra the time is divided as Visarga and Adana kaala.

Visargakaala

वर्षाद्शरध्हेमन्तेषुतुदक्शिणभिमुखेअर्के... शशिनिचाव्याहतबले...तत्रबलमुपचीयते।Cha. Su.6/7

During Varsha, Sharad and Hemantarutu Moon will be dominant,so there will be Upachaya of Bala in all living beings as the Sun is weaker. From August to December months Madhura, Amla and Lavana rasa are potentiated and it increases the strength of living being.

Hence these months are suitable for conception. This is also called as Dakshinayana.

Adana Kaala

During Shishira, Vasanta,Grishmarutu due to the sharp rays of Sun living creatures become weak. Katu, Kashaya and Tikta rasa are potentiated and not suitable for conception.

Kaala and Arthava -Chakra

Menstrual cycle is also a physiology occurring in phases with different time duration. Artava chakra is classified into

- ArtavaSrava Kaala
- Ritu Kaala
- RituVyateeta Kaala

Artavasravakaala

Time duration of Artava kalais 3 to 5 days

मस्सन्निशिपस्च्वदाहार्तिपन्चरात्रानुभन्दि| cha. Chi. 30/225

मासेमासेगर्भकोष्टाअनुप्राप्यत्र्यहंप्रवर्तमानम्। A. S.1/10

The Artava collected for a whole month in the dhamanis of Artavavahasrotas comes out from the yoni for three consecutive days.

Rutu Kala

Duration of Rutukaala

ऋतुस्तुद्वादशरात्रंभवतिद्रुस्तार्तव: |S.Sha. 3/6

Rutukaala is considered as 12 days.

द्वादशरात्रमितिषोदशदिनेषुमध्येआध्यंदिनत्रयमन्तिम्चषोदशंयोनिसन्कोचदिनंनगणनीयम्।

Su.sha. 3/6 Dalhana

Acharyas opine that the fertile period extends from 12 to 16 days after the Artavasravakaala. Dalhana explains it saying that among 16days of Rutukaala the first 3 days due to ArtavaSravanad last day due to GarbhashayaMukhaSankocha conception will not occur.

Rutuvyatita kala

During this period cervix gets constricted to entry of pumbeeja just like after sunset lotus becomes sankuchita, hence conception is not possible during this time.

Cause for failure conception during these days

नियतंदिवसेअतीतेसन्कुच्यत्यम्बुजंयथा।ऋतौव्यतीतेनार्यास्तुयोनि: संव्रियतेतथा॥ Su.Sha. 3/7

पद्मंसन्कोचमायातिदिनेअतीतेयथा,तथा।ऋतावतीतेयोनि: साशुक्रंनात: प्रतीच्छ्ःति॥ A. Sha.1/ 42

As the days proceedes after the rutukaala the garbhashyamukha becomes sankocha and prevents the entry of shukra just like the bloomed lotus closes as sun sets. Same way the sperm deposited after the ovulatory period will be fruitless as the cervix will be constricted during this period.

Time in relation to ovulation and conception: ovulation occurs approximately after 16 to 24 hours of LH surge hence 12 to 16 days after artava chakra is considered as fertile period the ovum can survive 72 hours after ovulation and sperm can survive for 72 hours in female genital tract.

Hence, we can say that fertile period will be of 120 hours same way spermatogenesis takes 60 to 63 days to complete and capacitation of sperm happens in 2 to 6 hours after it reaches the ovum

Implantation and time: after fertilization the cell division takes place and 2 cell stages completes by 30 hours embryo in morulla state (16 cell) reaches the uterine cavity and staysfreely on fourth and fifth day implantation happens on 6^{th} day so, time is major factor to decide conception and non-conception.

Kshetra

क्षेत्रमिवक्षेत्रं, तत्रशुक्ररुपबीजप्ररोहणात॥.... Cha.Chi. 2/1/4

BeejarupaSukra enters in to female hence she is called kshetra

यास्त्रिप्रहर्षिणितत्क्षेत्रंश्रेष्ठवाजीकरणम्इति। Chakrapaani teeka

Due to origin of progeny the female is called as kshetra, hence she considered as Agravajikarna.

In bhelasmahita Sarirasthana it is told that as the healthy apradustabeeja sown in well cleaned and ploughed field results in healthy crop, the Apradusta Beeja embedded in shudhasukra yields in Sreyasi Praja.

सुकुष्टक्षेत्रेबीजंप्रक्षिप्तंतत्रव्रीहि: वीहित्वायकल्पते, यवोयवत्वाय। (Bhela. Sha 8/2)

With all these references we can consider Kshetra as female reproductive system as a whole.

So, it is very well mentioned that only in shudha yoni conception is possible.

Kshetra in broad sense should be considered as streesharira as a whole

कन्यातुअतुल्यगोत्राम्असन्चरिरोगकुलप्रसुतां....अरोगप्रक्रुतिम्...(A.S.Sha.1/3).

Women should be Nirogi to have a healthy progeny. Women being a Kshetra should not be belong to a family where hereditary running disease is there. She should have stability of mind like integrity purity etc.

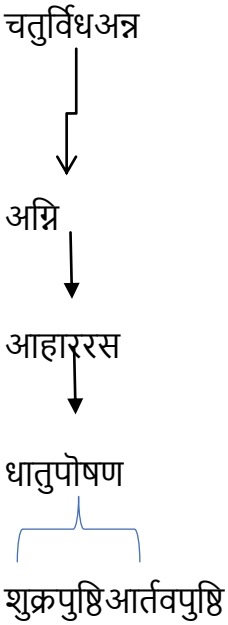
Healthy unvitiated reproductive system is the very much essential factor for the conception.

Woman with healthy reproductive system with proper physiology can produce healthy offspring. The healthy spermatozoa deposited in healthy vagina passing through healthy cervix with proper secretion enters the patent fallopian tube with normal ciliary actions unite with healthy ovum and produces the disease-free embryo.

AMBU - is fluid that nourishes embedded seed starting from day one throughout intra uterine life.

"अम्बुपुनराहारपाकजोव्यापिरसधातु: || Su.Sha. 2/33 dalhanateeka

Ambu is rasa dhatu formed after digestion



In normal physiology the rasa does Dharana, Tarpana, Snehana, Jeevana of body tissue by its Soumya guna. The chaturvidha anna, asita, petha, lidha, khadita having Sadrasa made of Panchamahabhuta when comes into contact with Agni forms the rasa after aaharapaka and Hridaya is the seat of rasa and from hridaya, rasa circulates through

the body through dhamanies by the action ovVyanaVata.

गर्भस्तुखलुरसनिमित्तामारुताध्माननिमित्ताचपरिव्रुधिर्भवति॥su sha.4/57-59

The fetus grows through rasa dhatu and vata hence, conception nature of conception qualities of progeny

'आहारशुध्दौसत्वशुध्धि:' (Chandopanishad)

Quality of mind depends on the quality of food taken.

यदन्नंभक्षयेन्नित्यम्जायतेताद्रशीप्रजा (vriddha Chanakya)

The diet consumed by mother decides the physical and mental condition of the baby.

BEEJA: Among GarbhasambhavaSamagri,

बीजंस्त्रीपुंसयोरार्तवशुक्रे।| Su Sha. 2/33 dalhanateeka

रक्ते- स्त्रिरजसि,शुध्दे,।तथा, शुक्रे- पुंबीजे; शुद्धे। A H 1/8 Arunadatta

Beeja is considered as male and female gametes.

In female Artava is essential for fertilization

स्त्रीणांगर्भोपयोगीस्यादर्तवंसर्वसम्मतं। (Bha.Pra. Purvakhanda 3/188)

Here Artava refers to Stribeeja (ovum). The type of Ankura depends on type of Beeja

याद्रशंतूप्यतेबीजंक्षेत्रेकालोपपादिते।ताद्रग्रोहतिततस्मिन्बीजंस्वैव्यर्जितंगुणै :।Manusmriti 8-36

For achievement of conception healthy oocyte and spermatozoa are essential.

शुद्धशुक्रार्तवंस्वस्थंसरक्तंमिथुनंमिथ: ।.... (AH Sh.1/18 Arunadatta)

Importance beeja in conception -

बीजस्यचैवयोन्याश्चबीजमुत्क्रुष्टमुच्यते।सर्वभूतप्रसूतिर्हिबीजलक्षणलक्षिताब्॥ Manu smr. 9/35

In Manusmriti it is mentioned that the Beeja is more important than the Kshetra as the progeny will possesses the qualities of Beeja embedded and not that of the field.

The Beeja formed by the Soumya bhava of the Rasa gets Agneyatwa after undergoing Dhatu paaka by the influence of Pitta..... 'आर्तवंआग्नेयं।'Su. Sha.3/3

Any abnormalities in Beeja,Beejabhaga,Beejabhagaavayava results in genetic abnormalitirs in the progeny, Abeejatha or anovulation may be one of such pathology which could be genetic inheritant.

Importance of Vata in Conception

शुद्धेगर्भाशयेमार्गे... अनिले.... (AH Sh.1/18)

... कर्तागर्भाक्रुतीनाम्।| चरक

According to VagbhataVata is one of the Garbhasambhavasamagri. PrakrutaVata is responsible for Beejotsarga, Shukrajanana, garbhaadaana, Vibhajana and complete

GarbhaVridhi.

"तत्रस्त्रीपुंसयो:संयोगेतेज: शरीराद्वायुरुदीरयति,

तस्तेजोअनिलसन्निपाताच्चुक्रंच्युतंयोनिमभिप्रतिपद्ध्यतेसंसृज्यतेचार्तवेन" **॥ (**Su.SH.3/3)

During the coitus heat generated stimulate Vata then Sukra is ejaculated by the combined effect of Tejas and Vaayu into the Yoni, unite with Artava and forms the Garbha.

ग्रुह्णातिवायुर्यस्यांचयोनौशुक्रमुपागत:।बिबर्तिगर्भिणिगर्भंशुद्धार्तवसमन्विता॥ (Bhela.Sha 3/6)

Vata does the BeejaGrahana in Yoni and carries it to Artava and Garbha is formed so, Vata is essential for process of conception in balanced state. As reproductive organs are located in Apanavatasthana, it governs the functions of those organs. It is also said that without Vata the Yoni never gets diseased.

Abnormal Vata leads to Vandhyatwa by disturbing Artava, Sukra and Yoni. Hence Vandyatwa is considered one among 80 Vatajananatmajavyadhi.

Importance of Satwa in Conception

For achievement of conception mind should be well balanced it should be satvik.

.....सौमनस्यम्गर्भाधारणं Ca.Su.25/40

गर्भारम्भकशुक्रशोणितसंसर्गकालेमातु: पितुश्चयद्गुणबहुलंतयोपुत्रदुहित्रोर्मन: ||

Ca.sha.8/12, Gangadhar)

Satwa of the progeny depends on the psychological state of parents at the time of Garbhaadhaana. Hence the garbha will carry satwika, raajsika and tamasika mano gunas according to the dominance in the parents.

Importance of Satmya in Conception

Is one among Sadbhavas it means wholesome dietary practice.

यावत्खल्वसात्म्यसेविनांस्त्रिपुरुषाणांत्रयोदोषा:प्रकुपिता:

शरीरंउपसर्पन्तोनशुक्रशोणितगर्भाशयौपघातायौपपद्ध्यन्ते ,

तावत्सम्र्थागर्भजननायभवन्ति। (Cha. Sha 3/11)

Parents can reproduce offspring when their sukra, artava , garbhashaya are not vitiated by dusita three dosa caused by practisingastamyaaharas.Bysatmya one can give virya, bala, arogya, varna medha, hence conception can be possible if both the couple are practicing satmyaahara and vihara.

Importance of Atma in Conception

It is Jeevatma which enters the Garbha after union of SukraShonita.

भूतैश्चतुर्भि : सहित: सूक्ष्मैर्मनोजवोदेहमुपैतिदेहात्।

कर्मात्मकत्वान्नतुतस्यद्रुश्यंदिव्यंविनादर्शनमस्तिरुपम्॥ (Ch.Sha.2.31)

The atma with satva and four bhutas (except akasha) leaving the whole body enters to the new garbha and is invisible and can be perceived only by divine power just like rays radiating from sun, fire is unperceivable.

The Shubha and Ashubahapoorva karma phaladecide the quality of future body. Prana, Apana, Prerana, Dharana, Akruti, Swara, Varna, Sukah , Dhuka, Ichha, Dvesa, Chetana, Driti, Bhudhi, Smriti, Ahnakara, Prayatna, all are the contribution of Atma to Garbha.

So, after SukraShonitaSamyogaenrty of Atma along with Satva is very much necessary to form the Garbha. Hence Embryo is formed out of the combination of the all these factors.

Abnormalities of any of these factors leave the woman infertile.

Essential factors for conception include following,

शुक्रासृगात्माशयकालसम्पद्यस्योपचारश्चहितैस्तथाऽन्नैः||

गर्भश्चकालेचसुखीसुखंचसञ्जायतेसम्परिपूर्णदेहः॥च.शा.२/६

For the conception to happen in time with healthy embriyo, normalcy of Shukra, Asruk, aatma,Ashaya needed. During Nisheka and garbhaavasthaproperupachara should be done with hitaaharaandvihara. By this the Garbha with healthy mental physical body take birth intime.Artava as ovarian hormones.Ovarian hormones play vital role in functioning ofreproductive system.

बालानापिवय: परिणामात्शुक्रप्राधुर्भावोभवति,रोमराज्यादयश्चविशेषनारीणाम्।su.su.14/18

In males Shukra appears after the specific time and ingirls Artava and Stanya appears after specific time and she gets Romaraaji.

This refers to appearence of secondary sexual characters by the influence ofovarian hormones. Hence in this context we can consider artava as ovarianhormone.

Role of Tridosha on Female Reproductive System

Vata–वागतिगन्धनयोःइति(Su. Su. 21/5) that which is responsible for the movement is called as Vata. Stanas of vata are paksvashsya, kati,saktiand others ,where the main organs ofreproductive system like uterus is situated . The initiation of menstruation at menarche is by Vata and the menstrual blood collected throughout the dhamanis is expelled by the prakrutaapanavata, as far as the ovulation is concerned, rupture of dominant follicle and escape of ovum is the function of vata.

Pitta- पित्तंतपसन्तापे॥(Su. Su. 21/5) pitta refers to which is hot in potency and gives heat to the body is termed as pitta. It is situated between the hrudaya and nabhipradesha. Nabhi is the special seat of pitta.

Kapha- श्लिशआलिन्गने(Su.Su. 21/5) .That which binds together is called as kapha.केनजलेनफलतिइतिकफ. Kapha is one of the products of water .In prakruthaavastakaphaistermedas the bala to the body and in the vikruthaavastha it is called as mala. The main sthanas of the kapha are uras, kanta, shirasmedaetc.shiras being the main site of kapha,regularity of H-P-O axis for menstrual cycle is the main function of kapha

Role of Vatain Various Stages of Womens Life

1. Garbha formation–शुद्धमार्गाशयेमार्गे.... अनले॥ (A.Hr.Sha 1/8)

Acc.to vagbhata, proper functioning of vata is one of the important garbha sambhava samagri for pregnancy.

2. वायुःविभजति-(su.sha 5/3)Vata is responsible for the maturation of gamets by cell division ." वायुर्हिकालसहित: शरिरंविभजतिसन्दधातिचेत्...Su.Sha 5/3 vata along with kala is responsible for division and association of gamets thus helping in formation of garbha
3. तेजयेनपचति-(Su.Sha 5/3)pitta is responsible for the transformation and pachana kriya taking place at tissue levels which helps in overall development of the garbha
4. आपःक्लेदयति, प्रुथ्विसंहनति,(Su.Sha 5/3) Role of vata in fertilization –The tejas (pitta) generated during the intercourse activates the vata and due to the combined action of vata and tejas(pitta) the shukra is ejaculated into the female tract where the fertilization takes place.

Role of vata in fetal nourishment-

व्यानेनरसधातुःहिविक्षिपोचितकर्मणा..... (A.Hr Sha. 3/68)

The growth of the foetus inside the womb is affected by the rasa dhatu formed by the aharaintaken by the women, which is circulated by the virtue of the vyanavata through the srotas of the mother. Thesesrotas are connected to the naabhinadi of the garbha. Vyanavata is responsible for the overall circulation among the body

Deprevation of the nutrients leads to garbhavyapat such as upavishtaka,nagodara and garbhashosha.

Role of tridosha in organogenesis –

1. Srotas, guda and basti is formed by the prasadabhaga of shonita andsleshma with pachana of pitta and intering of the vata into thetissue.Ushma and vata are the main factors responsible for the formation of the strotas.when this enters into the mamsa dhatu, it divides into peshi
2. Sira,snayu is formed by the sneha part of the medas, where the sira is formedby the mrudupaka and snayu is formed by the kharapaka.
3. The navarandras are formed by the action ofvata

Acc to haritha – panchamahabhuta forms a pinda, by paripaka it forms the ghanasanghata by the virtue of vyanavata and forms hasta, pada,shirasandavayava

1. Udanavata forms cavities in the upper part of thebody.
2. Apana vata forms cavities in the lower part of thebody.
3. Vata dosha spreading and influencing different regions of the bod givesshapes to perticlarpartsaccordingly.

2. The matruja bhavas such as budhi, medha, dhi, dairya, and smriti of garbhais obtained by the sadhaka pitta.

Role of tridosha in puberty –

Vata- Prana vata and vyanavata is responsible in maintaining the H-P-O axix. Hence a woman attains menarche and release of arthava monthly at regular intervals

Pitta-बालानामपिवयःपरिणामातशुक्रप्रादुर्भावोभवति।

रोमरज्यादयस्चविशेशनारीणम|| (Su.Su.14/18)

Shukraappears in the male and arthava in female .by the action of pitta the romaraji and other sexual charecters develops inwomen

Role of tridosha in menstrualcycle-

पाचितपित्ततापेनरस्सद्याधातवःक्रमात।

शुक्रत्वंयान्तिमासेनतथास्त्रीणंरजोभवेत।(Sharngadhara Poorva khanda 6/10)

under the influence of samanavata, good quality of arthava is formed . The aggrevatedrakta formed from the rasa is expelled every month under the action of apanavata.

The first phase of aarthavachkra is रजःस्रावकाल- rajas accumulated in garbhashaya

after rutukala is termed as the purana rajas. This accumulated rasa is eliminated from the garbhashaya during rajahkala. The predominant dosha being the vata during this phase of menstrual cycle.

The second phase is ऋतुकाल- this is most suitable period for achieving conception. This is the fertile period,mostly 12-16days from the 1st day of menses. Predominant dosha is kapha during this phase. kapha helps in regeneration and proliferation of the endometrial tissue.

The third phase is ऋतुव्यतितकाल- it is the 9-13thday after rutu kala. Constriction of yoni and purana rajasis formed during this stage. As lotus flower closes after sunset, similarly after rutu kala the yoni ofthe women constricts and does not permit the entry of the beeja(sperms) into the uterus. Pitta is predominant dosha during this phase. Pitta is responsible for the metabolic activities, and also development of secondary sexual charecters.

9. **During labour**-

Apana vata during labour is called as prasutamaruta, controls the mechanism of labour by uterine contractions and relaxation.Pitta dusti during labour leads to haemmorhage and other complications.

10. During suthikakala-

Vata dosha is soon accelerated after the child birth and women become emaciated and have shunyashareera. The madhurabhaga of the rasa dhatu circulating through the entire body by the action of vyanavata reaches the sthana and then excreates as sthanya. The balance of tridoshaisessential in soothikaavasta otherwise leads to many soothikavyadhis such as garbhinijwara, garbhinimakkala(vata+rakta)

11. **During menopause-**

तदार्त्वंपन्चशतंऊर्द्धं ..(Su Sha3/11 Dalhana)...menopause attained at the age of 50years, which is the period of vatavrudhiinawomens life. Slow deterioration of rajas and stanya takes place during this phase .

FACTORS RESPONSIBLE FOR INFERTILITY

Yoni pradosha

योनिप्रदोषान्मनसोऽभितापाच्छुक्रासृगाहारविहारदोषात् |

अकालयोगाद्बलसङ्क्षयाच्चगर्भंचिराद्विन्दतिसप्रजाऽपि || च.शा.२/ ७

The word "Yoni" refers to entire female reproductive system. Any abnormlities may lead to difficulty in conception or continuation of the pregnancy.

Under twenty Yonivyapads all most all of the gynaecological diseases are included. If they are not treated properly cause infertility (Abeejata).[13]

Few of the yonivyapads cause infertility either primary or secondary if not treated. They are,

Asruja Yonivyapath- A clinical condition with bleeding pervagina during early pregnancy leading to early pregnancy loss.This can be taken as secondary infertility due to implantation defect.

Vamini Yonivyapath

The feature of this yonivyapad is the expulsion of beeja orshukrawithartavafromthegarbhashayamukhabysixthdayorseventhdaysaftertheentryof Shukra into the Yoni. Here may be due to luteal phase defect implantation failure leaving the female infertile.

Putragni Yonivyapad- this condition is repeated abortion due toArtava dosha.

Shandi Yonivyapath- it is a congenital abnormal condition where the lady will not menstruate. This is due to beeja dosha.Over all in as the major dosha involved in the pathogenesis of Yonivyapth, as upadrava the vitiated yoni will not accept the Beeja and the female with such yoni becomes infertile.

Aticharana Yoni Yyapada

Acharya Sushruta says that this disease is caused by excessive coitus. The woman does not achieve conception. Charaka and Vagbhatta have described it to be Vataja, while Sushruta due to Kaphaja. In the initial stage, due to intense sexual desire, the woman may feel vaginal itching and due to repeated coitus may have excessive mucoid unctuous secretion from cervical and endometrial glands, which are the clinical features of Kapha as explained by Sushruta. Bhavaprakasha has explained that in this condition the woman discharges Raja before the ejaculation of male partner. It can be taken as vaginitis due to excessive coitus associated with infertility.

Trauma as a Cause of Infertility-

Injury to Artavavaha Srotas-

Acharya Sushruta has included Vandhyatva, dyspareunia and amenorrhoea (anovulation). under the consequences of injury to Artavavaha Srotas .[14]

clinically durig dilatation and curratage excessive curatage of endometrium leads to damage to the basal layer of the endometrium causing permanent amenorrhoea the condition is called Asherman's syndrome that the female lossed the capacity of reproduction. During ruptured tubal pregnancy due to the damage of tubes natural

pregnancy is not possible.

Yoniarsha

Yoniarsha which is caused by the vitiation of Tridosha obstruct the passage for shukra and leads to infertility by destroying the Artava.[15]

Garbhakosha Bhanga

Is refers to the retro-displacement or prolapse of uterus which leads to infertility. Among the many causes of infertility, the Garbhaposha –bhanga in which semen fails to deposit in the vagina as there is prolapse of uterus. [16]

Sphalita Mutratva

Sphalita mutratva in girls is also responsible for Vandhyatva [17]i.e., due to gonorrheal infection there is obstruction &inflammation of urethra, as these gonococci infection ascends upwards along the urinary system. And gonococcal causes salphingitis, urethritis and endometritis.

Utkshipta Yoni

Due to retro- flexion the cervix is displaced upwards and sperm is unable to pass through cervical cavity.

Mano- Abhitapa

Due to physochological factor many couples suffers from anxiety depression which disrupts harmony between them and due to lack of proper sex education they don't know the exact fertile period and it affects the fertility rate[18] Intercourse with a psychologically disturbed lady (afraid, sorrowfull, angry, unwilling for intercourse, with an intense of sexual urge or lady with digestive disturbances becomes a futile one.[19] Due to dharaneeyavegas , shokaVata will be vitiated such as Bhaya, Shoka, Krodha, Lajja. So, it increases hypothalamic activity of CRH (corticotrophin releasing hormone) and further it inhibits normal GnRH pulsatile secretion and ultimately anovulatory cycles occur.

Beejadushti

When in Ovum, the gene concerned with uterus is damaged, the progeny becomes sterile.[20] Any variation in the sperm count and qualities of sperms such as viscosity, pH cuases infertility. Pitruja bhava such as kasha, danta,loma,nakha, asthi etc. are imbibed into the fetus through sperms. [21]

Artava Dushti

वातपित्त श्लेष्मा शोणित कुणपगन्धि पूतिपूय क्षीणमूत्र पुरीष रेतसः प्रजोत्पादने न समर्था भ्वति ।
(Su.Sha. 2/5)

The word Artava is considered as ovum, menstrual blood, and ovarian hormones according to different contexts. Artava vitiated by different doshas produce infertility due to destruction on beejarupi artava.[22] Abnormal folliculogenesis results in menstrual abnormalities and anovulation.

Ahara Dosha

Ahara dosha causes agnimandyata which causes rasa kshaya finally leading to dhatu kshaya impairs hormonal function. [23]

Diet taken without following the Ahara vidhividhayatana explained in the science influence nourishment of the body by Santarpana or Apatarpana which influences normal secretion of hormones there by disturbing the process of ovulation and hinders conception. In modern society people are depending more on artificial food with preservatives, colouring agents, taste makers etc with less nutritive value as well as they have more harmful thing making the people sick and affecting in different aspects of health like reproduction.

A reaserch study shown that genetically modified foods, according to researchers, are becoming a real problem when it comes to fertility, causing an influx in worldwide infertility rates. Female animals being studied showed an alarming increase in an inability to get pregnant as well as a spike in premature births; low birth weight babies and infant mortality after being fed a regular diet of GMO.[24] Women who eat lots of low-fat dairyproducts face an 85 percent higher risk of ovulatory infertility than women who consume little or no low-fat dairy products.[25]

Vihara Dosha

1. Ativyayama
2. Atimathuna
3. Ratrijagarana
4. Diwaswapna

These types of mode of life affect the metabolism (Agni) and vitiates the Tridoshas causing yonivyapaths and Artavavyapaths leading to infertility.

A research article proven that a woman who consumes at least one alcoholic beverage per day have a nearly 50 percent greater risk of ovulatory infertility than women who

drink no alcohol.[26]

One more study says, Women who are exposed to large quantities of perfluorooctanoic acid (PFOA), a synthetic chemical used in the linings of microwave-popcorn bags, facean infertility risk ranging from 70 percent to 134 percent higher than that of women with the least amount of PFOA exposure.[27]

Akala Yoga

If the couple are unaware of the fertile period and trying for conception randomly are going to be infertile due to ignorance and during teenage and elderly females' conception is not easy and also continuation of pregnancy with normal clinical outcome is very difficult sating that age is very important factor to be considered as the conception is concerned.

A prospective cohort study done by collecting the data from 7 European countries shown that the pregnancy rates decrease steadily with increasing age of the woman, causing an increase with age in the average time to pregnancy. The proportion of women failing to conceive within 12 cycles (thus meeting the criterion for clinical infertility) ranges from 8% for 19- to 26-year- olds to 13–14% for 27- to 34-year-olds, to 18% for 35- to 39-year-olds.

Nidana for Vandyatwa Due to Abeejatha- Anovulation

We get scattered references available for anovulation as Beejopaghata, Pushpopaghata and Abeejatva.

Nastartva

Due to aharaja and viharaja nidanasVata gets aggravated and causes "Rasa Dhatu-Kshaya" Because of this Dhatu Kshaya,which causes the Kshaya of its Upadhatu Beejarupiartava as well as Masanumasika Sravarupi Artava. It means there will be anovulation and menstrual irregularity.

Artavavaha Strotovighata[28]

Anulomagati of vata is responsible for ovulation, any trauma/injury to the varies or ovarian blood vessels causes vitiation of Vata followed by Sangha and Upadhatu Kshaya. Arthavanasha is cuased by vitiated Vata and further causing Arthava Nasha.

Revati Jataharini (Pushpaghni)[29]

Acc to Acharya Kashyapa the women with regular menstrual cycle are called as Pushpangni. but is without the Beejarupi Pushpa. Along with this the lady also will have Lomashaganda and Sthula. The cause of initiation of this Jataharini is Adharma

in indulging diet as well as life style along with psychological disturbances .This causes Sanga in the Srotas that turns into Vikriti like anovulation. Therefore, it can be considered as a Sanga Pradhanavikara.

Avarana[30]

The Prakupita Kapha due to its nidana does the Avarana of Apana vata leading to different pathogenesis like loss of function like Artava Nishkramana Kriya and also Beejarupi Artava Nirmana.

Vandhya Yoni Vyapada[31]: According to Sushrutha Arthavam can be considered as ovum and anovolatory cycles can be considered as Vandhya yoni vyapat. All yoni vyapat is caused dur to vitiation of vata

Use of tikshna virechana in mridu kostha

According to Acharya kashyapa, if TikshnaVirechana is given in Mridukostha woman, Vata gets aggravated and causes Beejopaghata[32]. Due to vitiation of Apanavayu, it prevents the rupture of ovarian follicle causing Beejopaghata.

Beejadushti

During Garbhavastha, if mother takes Vata PrakopakaAhara and practices Vata prakopa Vihara and the female fetus is affected with vitiated vayu then her Beeja, Beejabhaga and Beejabhagaavayava can be vitiated and can manifest congenital abnormalities in female reproductive organs.[33]

Dietetic Habit

Due to consumption of junk foods and following improper dietic habits the Beeja may get vitiated [34]. Following the abnormal dietic habits like Vishamashana, Adhyashana, Anashana, Viruddhaannapana causes Agnivaishamya and Rasadushti leads to Artavadushti ends with Beejarupi Artavadushti in the form of Anovulation.

Purvarupa

There is no description of premonitory symptoms ie. Purvaroopa of Vandyatwa in any of our classical textbooks by our acharyas. Acharya kashyapa has mentioned Vandhya yoni in the decription of Vataja-nanatmajavyaadhi .

Rupa: "Vandhyanashtartavamvidyat" (Su.Ut 37/10-11)A woman, in whom Artava has been destroyed, is termed as Vandhya. [35]

Management of Vandyatwa-

स्नेह स्वेदवमनविरेचनास्थापनानुवासनैः क्रमशः उपचरेन्मधुरौषध सिद्धाभ्यां क्षीरघ्रुतपुष्टं पुरुषं, स्त्रीयं तु तैलमांसाभ्यामित्येके. (का. सं. शा 5/3)

In classics Acharyas have described Nidana and Chikitsa for Vandhyatva at different contexts.the treatment has been given according to the causative factors. The Vandhyatva chikitsa includes- treating the underlying pathological condition of infertility, Avoiding the etiological factors (Nidana parivarjana), basic treatment methods of Vandhyatva by Garbhapradayogas, following regimens indicated in Garbhaadhana.

1. **Nidana Parivarjana**

Samkshepatahakriyayogonidanasyaparivarjanam (su.u.1/25)Infertility is a condition caused by different etilogical factors. Identifying those causes and strictly avoiding them is the first and foremost thing in the treatment.

2. **Treating the Underlying pathology**

The pathology should be identified and treated accordingly, Treatment for Asrukdosha[34] ·Panchakarma- Doshanusaravamanadiprayoga, Sthanikachikitsa- Kalka, Pichu, Yoniprakshalana.

3. **Shukradosha hara Chikitsa**

Rasayana, Vajeekarana, Mutra rogaharadravyas

Treatment For yonivyapad[37]

After proper Purvakarma Panchakarma Chikitsa should be given. As vata is the prime cause for Yonivyapath, without vata vitiation no yonirogas will manifest, that should be controlled well. Application of Lavana Taila, Swedana with Pindasweda and Kumbhikasweda, Parisheka with Sukhoshnajala, Vataharaahara and according to the condition after Shodhana Uttarabasti can be administerd.

Treatment of Anartava[38]

In a condition of Artava Nasha Acharya kashyapa mentioned use of Shatavari – Shatapushpa .by use of this Vandhya or even Shanda can get a son.

Regimens Indicated in Garbhadhana

As Purvasamyogavidhi some regimens are told, i.e Shodhana, maintainance of Sadvrutta, avoiding negative emotions.[39] By proper purification and Samskara Yoni, Garbhashaya, Beeja and Manas will remain unvitiated and are ensured leading to

healthy pregnancy by perfect unification of Beeja.[40]

Panchakarmas in Vandhyatva[41]

The infertile women should be prescribed Vamana, Virechana and Asthapanabasti by which she conceives.

1. **Vamana**

Given for Kapha Dosha Nirhana , Vamana does the Soumya Dhatu Shaman and ignites the Agni Dhatus in the body which helps in Pitta Vrudhi and inturn increases the quantity and quality of Arthava in the stree.

2. **Virechana**

Acc to Kashyapasamhitha for Akarmanyabeeja which is considered as anovulation, Virechana is considered as the best treatment.

3. **Basti**

- Niruhabasti is considered as amrutha for an infertile woman.
- Anuvasanabasti is an ideal treatment in Beeja Doshasambandhi Vandhyatva Yapanabasti is very ideal in Streevandhyatva.
- In cases of Beeja Dosha Vandyatwa, like Alpadosha, Nastaarthava and Nastabeeja Anuvasana Vasti is ideal.
- Yapanabasti performs both the Niruha basti and Anuvasana Basti which does both Snehana and Shodanakarma.

4. **NASYA**– The medications administered through the nasal route reaches the shiras and helps in pulsatile action of gonadotrophin releasing harmones and promotes the ovulation. Thus, helping in treatment of infertility. Ashwagandha Siddaksheerapaka every day in morning hours after Rutusnana Lakshmana mula uprooted in Pushya Nakshatra, pounded with milk Lakshmanakalka with ghee or milk for Nasya.

Other yogas[42]

Narayana Taila, Shatavari Taila, Phala Ghrita, Lasuna Ghrita, Shatavari Ghrita, Kalyanaka Ghrita, Kushmandaavaleha

Prajasthapana Gana- Aindri, Brahmi, shatverrya, Sahasraveerya, Amogha, Avyatha, Shiva , Arista, Vatyapushpi, Vishwaksenakanta

Charaka mentions Prajasthapanagana. These 10drugs have the quality of attaining conception and combat infertility.

Pippalyadi Yoga[43]

पिप्पलि श्रुन्गवेरश्च मरिचं नागकेशरं ।

घ्रुतेन सह पानेन वन्ध्यापि लभते सुतम् ॥ 52 || Bhai ra. Yoni RogaChikitsa.

This is a special yoga mentioned in Bhaishjya Ratnavali for treating Vandyatwa. The same Yoga is selected for the study.

Samprapti Bhedana

Probable Samprapti: Due to various Aharaja, Viharaja and Manasikanidanas, Kaphaprakopa does the Agni Sameepasta Samana Vata Dusti leading to Jaataragni Mandhya. Ama Utpatti takes place from which Rasa Dhatu Dusti and Uttarotara Dhatu Dusti takes place.

Due to Khavaigunya in the Dimba there is Arthavasrothavrodha and Srotho sangha. Beejarupi Arthava Nasha takes place leading to Nastarthava.

Samprapti Ghatakas

Dosha: Vata Pradhana Tridosha.

Dushya: Rasa, Rakta, Artavava.

Srotas: -Rrasavaha, Artavavaha.

Srotodushti: Sanga

Udbhavasthana: Amashaya, Pakvashaya

Sancharasthana: Sarvashareera

Vyaktasthana: Phalasrotus

Rogamarga: Abhyantara

Sadhyasadhyata: KrichraSadhya

Samprapti Of Nashtarthava Leading to Vandhyatwa

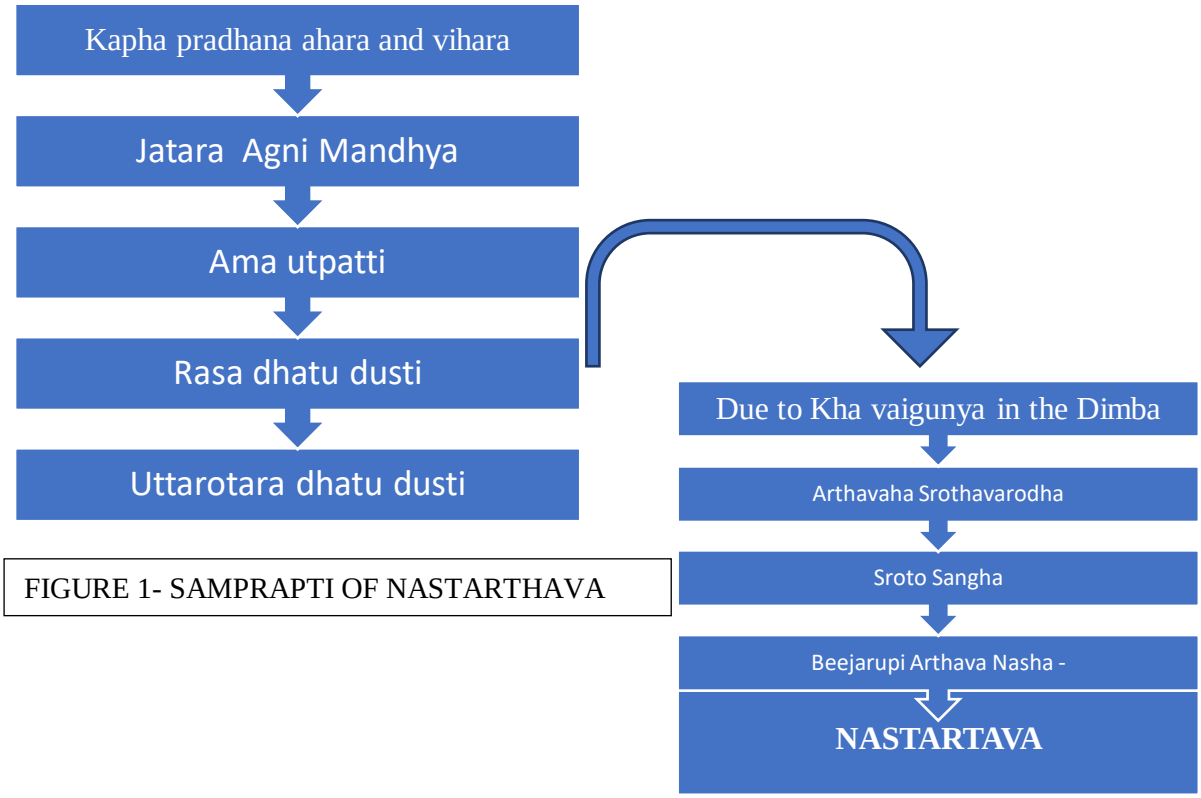


FIGURE 1- SAMPRAPTI OF NASTARTHAVA

SAMPRAPTI BHEDANA

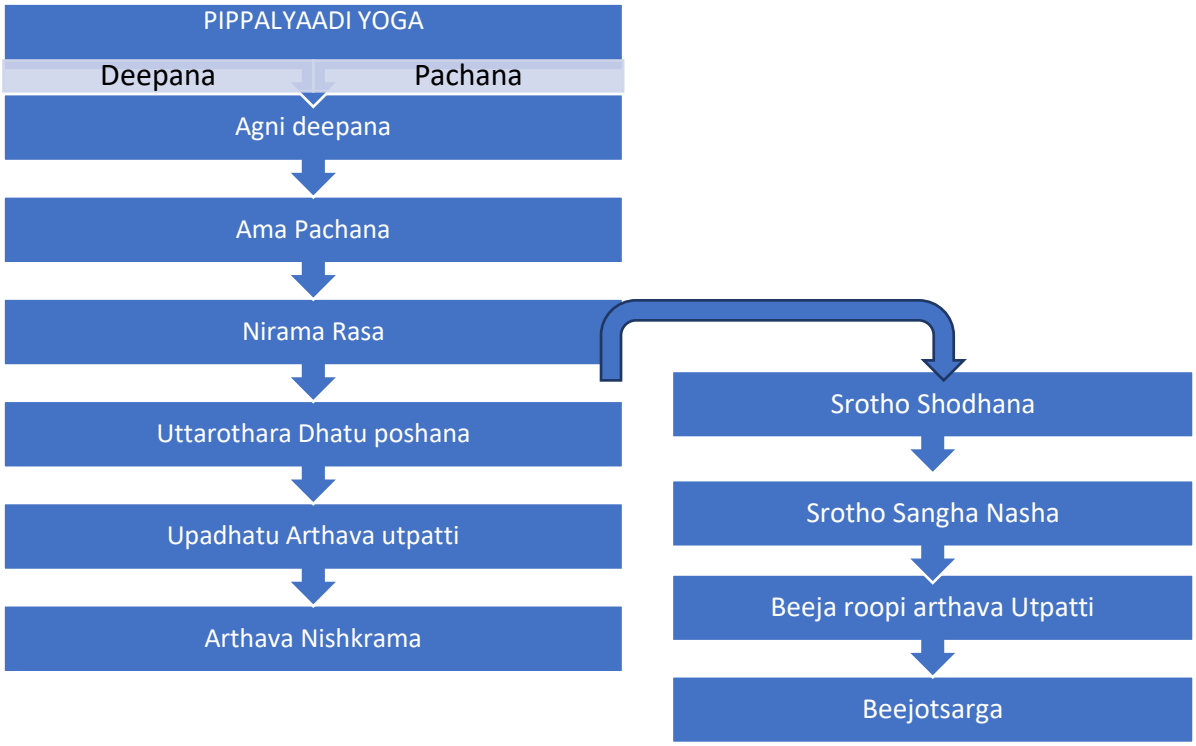


Figure 2- Samprapti Bhedana

Pippalyadi yoga with Drugs such as Pippali, Maricha,Shunti and nagakeshara has both Deepana and Pachana properties which does Agni Deepana and Ama Pachana

Due to Ama Pachana there is niramikarana of Rasa dhatu and uttarothara dhatu Poshana and UttarotaraArthavautpatti and arthavanishkramana takes place.

Ama pachana also does srotoshodhaka and Sroto Sangha Nasha wherein the beejaroopiarthavautpatti takes place leading to Beejotsarga.

5.2 MODERN REVIEW ON DISEASE

Anatomy And Physiology of Ovary in Human

There are two ovaries in the reproductive system of female present in the pelvic cavity which weigh about 5-10 gms. They are attached to the broad ligament on the both lateral side of uterus by mesovarian. Both the ovaries are attached by the ovarian ligament and are suspended from the cornua of the uterus. The thickness of ovary is 0.5-1.5cms. Usually, the right ovary will be larger than the left.[44] The development of follicles, release of steroid hormones, maturation of germ cells, ovulation are the functions related to ovary. The surface of the ovary is corrugated and pale. If the corpus luteum is formed after ovulation, and maintained after fertilization, the surface of ovary looks yellow at this site. The ovary is not covered by general peritoneum. All the vessels and nerves suppling to the ovary enter and exit through the hilum attached to the mesovarium. The hilus cells are homologous to the interstitial cells of the testis.On the cross section of ovary, we can observe two different zones by name Medulla and cortex. The medulla is the inner zone which contains spiral vessels and is more vascular, where as the cortex is the outer zone.

Medulla and cortex are made up of connective tissue stroma with blood vessels and nerves. The follicles of varying sizes with their derivatives along with corpora albicantia and corpora lutea will be present in medulla and cortex. But the primordial follicles are usually found in the cortex. During early life the cortex is covered by germinal epithelium which has single layer of low cuboidal cells. Afterwards the connective tissue layer, tunica albuginea is formed covering the ovary.

Changes in the ovary with age and parity – During infant stage and childhood, the ovaries are small, elongated structures having smooth surface and contains Primary oocytes. In Post-menopausal age, ovaries reduce in size due to atrophic medulla. In advanced age the ovarian surface becomes smooth as that of childhood. There will not be any follicles remain in the cortex.

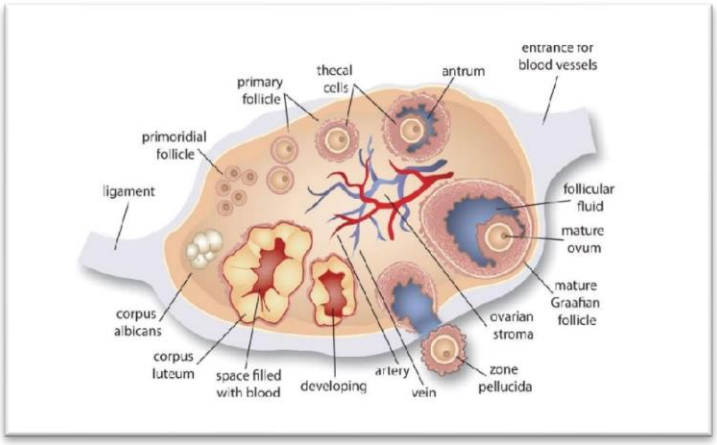


FIGURE 3 – ANATOMY OF OVARY

Physiology

Functions of ovary

The functions of ovary are under the regulation of HPO axis. Both ovaries are having similar function and one ovum is released at a time alternatively by each ovary.

- Steroidogenesis/ female sex hormones /ovarian hormones
- Oogenesis

These functions are controlled by the HPO axis by endocrine, paracrine and autocrine glands

Physiology of Ovulation[45]

At birth, the ovary of the female child contains all the primary oocytes which is scattered among the mesenchyme stroma cells in the middle medulla and cortex area. Total of 2 million oocytes are present at birth. It is further reduced during childhood and at puberty it is about 3-5lakhs. Among those less than 500 of them matures in lifetime and will be lost by degenerative process. Each primordial follicle contains an oocyte and is covered by single layer of the granulosa cells, and differentiated stromal cells.

Following the birth, follicles which develop from the layers of granulosa cells undergoes luteinization and forms liquor follicular changes are due to the placental gonadotrophins and they disappear during infantstage. Sometimes due to presence of multi ovular follicles in adult life there are chances of multiple pregnancy.

In childhood because of the growing stroma, the ovaries grow in size and is quiescent and these ovaries fails to ripen and becomes blighted.Ovum is formed from the secondary oocyte from the ovary during ovulation, they first shed during menstruation and ovulation and is not regulated until 2-3yrs after attaining menarche.

This continuous until 45-50yrs and gets irregularafter 40yrs of life.

Ovulation usually occurs proceeding menstruation and even after cessation of menstrual periods in rare cases.

Ovarian cycle- Can be explained in 2 phases for 28days cycle. The ripening of the ovary during 1st14days of the cycle during the follicular Phase where the formation, function and activity takes place. Later during 2nd half of 14days where the function and degeneration of the corpus luteum called as the Luteal phase. These two phases are divided by the ovulation. The duration of luteal phase is constant and usually 14 +/- 2 days

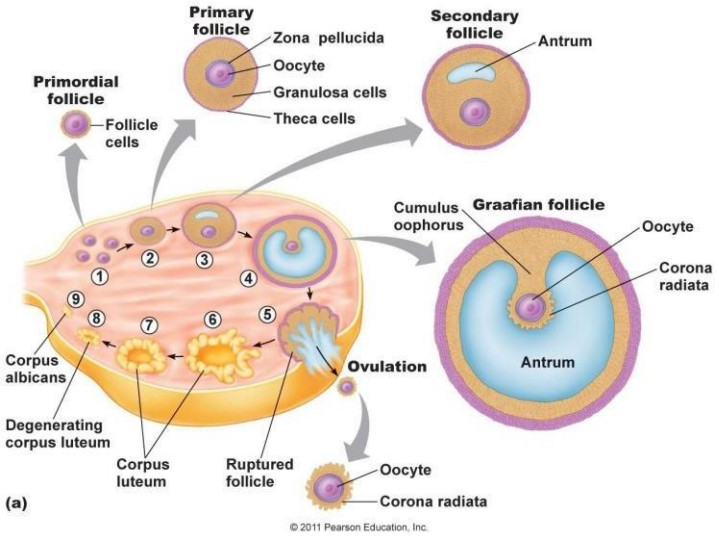


Figure 4– Ovarian Cycle

Stages of production of ova

Primordial follicle – In the beginning during each cycle there will be recruitment of group of primordial follicles, without the influence of gonadotrophins. The follicles get differentiated and grows due to stimulation of FSH. Hence the process will be transferred to gonadotrophin dependent stage. First of all, there will be growth of the oocyte. The single layer of granulose cells in the follicle expands and becomes multilayered cuboidal cells.

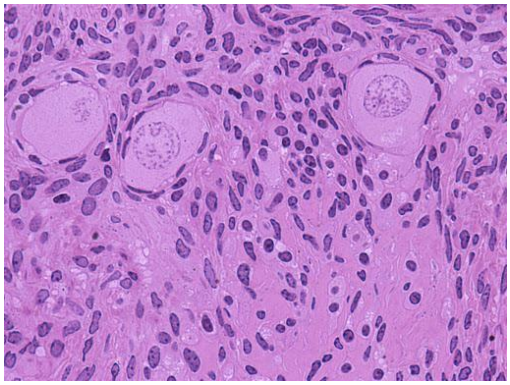


Figure 5 – Primordial follicle

Pre antral follicle: by the stimulation of gonadotrophin FSH the follicles continue growing. In the follicles the expanding Zona pellucida gets separated from the granulose cells which are surrounding the follicle. Continuousmitotic proliferation of granulose cells and theca cells at the same time occurs during when the primordial follicle becomes pre antral follicle. During the proliferation of granulose cells and theca cells estrogensecreted. After the maturation of dominant follicle into graafian follicle other follicles undergoatresia.

Two-Cell –Two Gonadotrophin Theory

According to this theory of follicular development sub division and compartmentalization of synthesis of steroid hormones takes place. In specific receptors of the cells of granulose cells FSH stimulation promotes the aromatase function for the production of estrogen.

At the time of steroidogenesis, the several enzymes and androgen synthesize by the theca cells having LH receptors to maintain the synergetic relationship.

Androstenedione an androgen is produced in the theca cells by LH stimulation. In the granulose cell FSH stimulates the aromatization for estrogen release. This microenvironment inside the follicle is needed for its nutrition and growth. Then differentiation and proliferation occur by the stimulation of FSH and other local hormones.

FOLLICULAR PHASE –

- Recruitment
- Maturation

Dominant are the three-phase involved in this phase

The recruitment or the follicles start few weeks before and one follicle takes the dominance. For this many follicles are get activated and get the selection of one dominant follicle and other become atretic. The androgen does two different regulatory functions at different concentration.

Low concentration -stimulates aromatization by having receptors in granulose cells. Higher concentration - The 5 alpha reductase activities will be intense to convert androgen to non-aromatizationform.

The expression of FSH receptors in the granulose cells are inhibited by the androgen pathway. Thisinturnreducesthe aromatize activity inside granulose cells leading to follicular atresia. So, this negative feedback causes less secretion of FSH. From HPO axis there by decreasing serum FSH level and leads to increase in estrogen level at periphery. Also, the inhibin is produced in excess in the ovary causing further decrease in FSH production. So, this fall in level of FSH is a discourage stage for the follicular development. As the dominant follicle has the most receptors for the estrogen, as it is growing estrogen will be continuously secreted. This increased level of estrogen still lowers the level of FSH. So other follicles will not get FSH to grow and get atretic. Just prior to ovulation LH surge takes place and ovulation takes place. As the size of ovum and its nucleus increases, the ripening of follicle is held. Then occurs cuboidal multi-layer covering by the two granulose cells and a colorless alkaline fluid with protein, fat and less quantity of hormones is secreted and become like a pool and separates the group of cells. As the fluid increase there will be formation take and graafian follicle is developed. This cyst like structure is lined by granulosa cells and called as membrane granulose and project into the cyst as cumulus oophores. This clump of cells has ovum which is covered by the zona pellucid which is made up of glycoproteins and are pale colored and is non cellular. Perivitelline space will be there between ovum and zonapallucida.

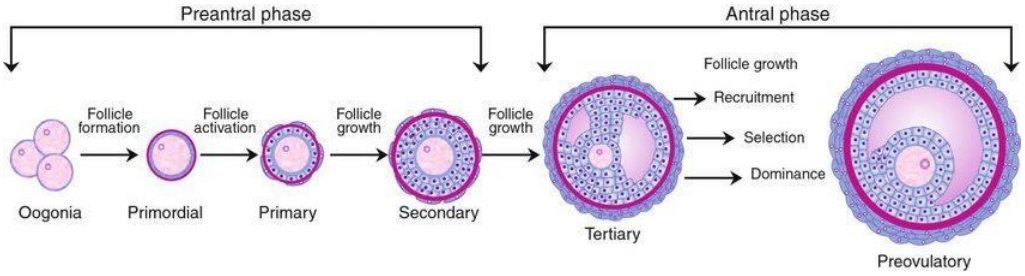


Figure 6 – Stages of follicular development

Corona radiate covers the granulosa cells, outside the membrane granulosa and the stroma cells become differentiated possibly as a result of a stimulus provided by vascular layer of granulosa cells is called theca interna which is spindle in shaped cells, surrounded by outside with stroma cells which compressed and modified and form a false capsule, which is called the theca externa.

The development of graafian follicle is asymmetrical and seen in the form of cone shaped theca interna which forms the surface of the ovary during ripening stage, which easily passes the tunica albuginea. It thus arranges itself so that the discus proligerus with the ovum lies on the side of the follicle adjacent to the peritoneal cavity.

Follicular ripening takes place with major histological changes within the last few hours or days of first 14days. The size of immature follicle is 0.03 mm in diameter and A ripen follicle size is 5 to 8 mm in diameter and it may reach up to 16 to 24mm immediately before rupture of follicle because of its large size it is visible to the nakedeye.

A fluid filled antrum which is composed of plasma and granulosa cell secretions are characteristics of preovulatory follicles. The oocyte remains attached to the follicle by a stalk of specialized granulosa which is called as the cumulus oophores.

Maturation of the Ovum

Oocyte is arrested in the meiotic phase and Meiosis is commonly divided into 4 phases – Prophase, metaphase, Anaphase, telophase. The prophase is further divided into Leptotene, Zygotene, Pachytene, Diplotene and diakinesis. After oogenesis only one final daughter cell is formed from each precursor cell and with excess genetic material 3 polar bodies are formed which are furtherdiscarded.

- Primary oocyte – When the oogonia develops and enters the meiotic prophase 1 it is termed as primary oocyte. This process begins around 8weeks of gestation and

survives from being atretic and reaches the fetal ovary before birth. Meiosis is resumed soon after ovulation when the oocytes is arrested in heprophase. The mechanism for the mitotic stasis is oocyte maturation inhibitor produced by the granulosa cells. The primary oocytes in the ovary of the newborn baby are in the early stages of meiotic division and the process arrests in the late prophase stage and it remains dormant until follicular ripening is established.Due to LH surge in the mid cycle, meiosis is resumed and completes within 35-45hrs before the ovum leaves the follicle. during this stage the number of chromosomes in the nucleus is halved and results in formation of secondary oocyte and polarbody.

- **Secondary oocyte**- They lie in the perivitelline space of the oocyte. After ovulation particularly after fertilization the second division takes place which secondary oocyte is released

Rupture of the Follicle-Ovulation

Here the ovum is released from the follicle gradually and is surrounded by a corona radiat of variable thickness and the discharge from the ovum is oozed out. Ovulation occurs due to thinning a degeneration of the cyst wall and is associated with the production of proteolytic enzymes. Progesterone induced midcycle rise of FSH helps in release of oocytes from its follicle. Ovum exists out due to contractions of the micro muscle cells in the theca externa and stroma.

They are activated by Prostaglandins which are essential for follicle rupture. The ovarian content is increased by the action of Luteinizinghormone. Sometimes the follicular fluid comes out with the ovum and slight bleeding takes place in the site during ovulation when the follicles rupture. This approaches to the outer end of the fallopian tube and moves towards the ovaries near the fimbrial end then it is embraced by the ovarian fimbriae and picks up into the ampullary part of the tube. this pick-up action of fallopian tube is very much necessary for the fertilization. if fertilization does nottake place the ovum survives only 12024hrs and later degenerates in the tube and doesn't leave any traces behind. Ova have been recovered from the fimbria and from the lumen of the tube to 2-4days after ovulation and 4-5days after the ovulation which is incapable to fertilize.

Luteal phase

Following ovulation, there will be marked changes that occurs in the wall of the follicle with collapse of the cyst and luteinization of the lining cells.

Thisluteinization has the impact both on the granulose and the theca interna. Then the corpus luteum of 1-2cms will project from the surface of the ovary. A fluid which is rich in phospholipid, carotene is present inside the cells. till 3days of post ovulation, corpus luteum is supplied by the blood vessels and grows further. this development will complete by 5days.thereafter, in absence of fertilization it gets degenerated.

For the normal luteal function midcycle rise in FSH for the sufficient LH receptors, the progesterone secreted during luteal phase suppresses the growth of new follicle. After degeneration of corpus luteum there will be decrease in the level of estradiol, progesterone and inhibin. So FSH levels starts raising by the negative feedback on the pituitary. By this next follicular phase begins and whole process of ovulation repeats.

Ovarian steroidogenesis

The normal functioning ovary synthesizes and secretes the sex steroid hormones-estrogens, androgens and progesterone, in a precisely controlled pattern determined in

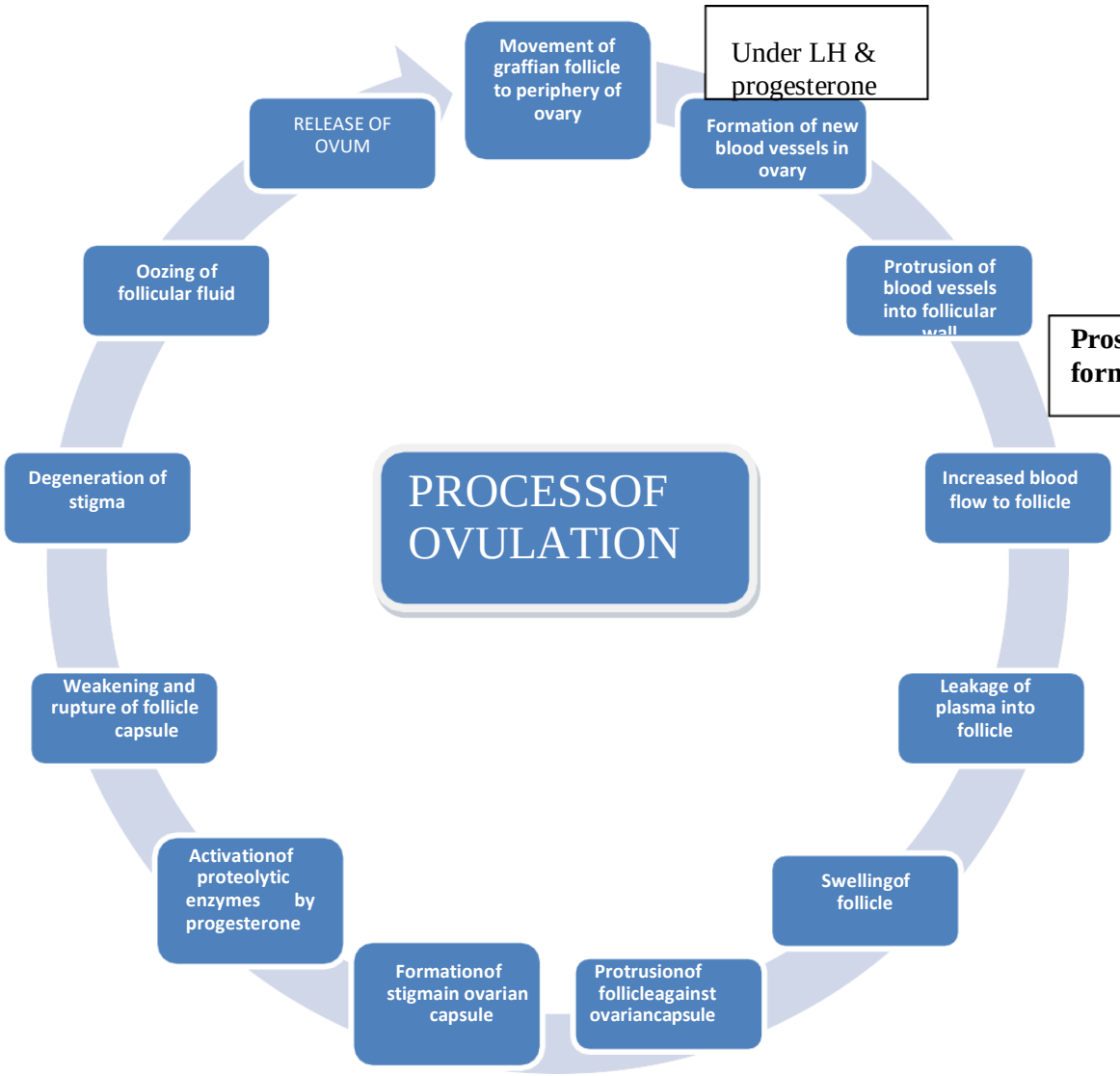


FIGURE 7 – Process of Ovulation

part by the pituitary gonadotrophins, FHS and LH the most important secretory products of ovarian steroid biosynthesis are progesterone and estradiol however, the ovary also secretes quantities of estrone, androstenedione, testosterone, and 17-hydroxyprogesterone. Sex steroid hormones play animportant role in the menstrual cycle by the preparing the uterus for implantation of the fertilized ovum. If implantation does not occur, ovarian steroidogenesis declines the endometrium degenerates and menstruation ensues.

Changes after ovulation:

After the ovulation there will be remarkable changes happening in the myometrium, fallopian tubes and cervix and vagina under the influence of hormones estrogen and progesterone secreted during and after ovulation.

1. In the uterus: the myometrial contractions increase due to the stimulation by estrogen and they come frequently.
2. The tubal musculature also has activity like uterine myometrium and there will be increased ciliary movement by the effect of serum estrogen which is necessary for the transport of ovum if fertilized into the uterine cavity.
3. After the ovulating there will be producedsecretion from the cervical glands which can be visible as vaginal discharge. this is again due to the presence of estrogen hormone. Hence the mucus can be stretched like threads of about 6.5cm-10-15cm. which can be taken as the ovulation indicator clinically and the test is called as spinbarkeit test. This state of cervical mucus is favorable for the penetration of the sperm in to the cervical os. During this phase the cervical os will be competent enough by the influence of Progesterone hormone.

Vaginal changes: The vaginal epithelium shows histological change

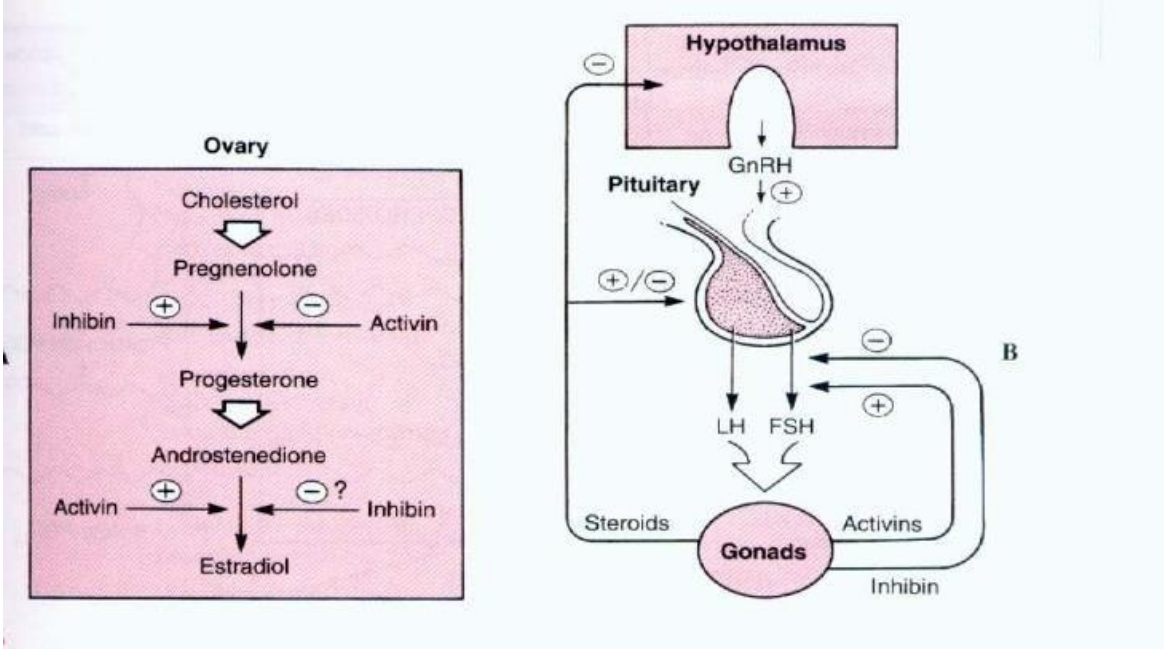


Figure 8 -Neuroendocrinology of reproductive system

REVIEW ON FEMALE REPRODUCTIVE SYSTEM IN FEMALE ALBINO RATS –[46]

The laboratory albino female rats are also like other placental animals which is featured by the intrinsic reproductive cycles and regular estrous cycles.The alterations in these reproductive tract cytology, physiology and histology are bought by the hypothalamus pituitary ovarian axis like in humans (HPO axis). The estrous cycle is divided into four stages namely the proestrus, estrus, meta estrus, di-estrous1and di-estrous 2.

Albino female Rats have cycles throughout the year and is called as polyestrous and the dioestrous phase is followed by the proeostrous phase of the next cycle and is said to be having continuous cycles. There is usually no gap observed in estrous cycles i.e., the anestrous. The estrous phase stops only during the lactation, pregnancy and pseudopregnancyphase. The albino female rats attain sexual maturity between 30-50yrs of their age. Kennedy and Mitra 1963 reports the average age of puberty in rats is on the basis of vaginal opening and the first estrus is on 38thday. aftee the vaginal opening the first oestrous cycle occurs and it continuous regularly for every 4- 5days for the variable proportion throughout its lifespan, depending on the strain of rat taken.

Rats demonstrates estrous cycle abnormalities at early 4-6months of its age and it is detected in sub chronic 90th day of toxicity studies.

According to the figure 2 the length of each stage of estrous cycle is demonstrated.

Mandl in 1951found out that in normal estrous cycle rats, the dioestrous phase showed greatest variation in duration and had highest influence over the oestrous cycle in terms of its length.

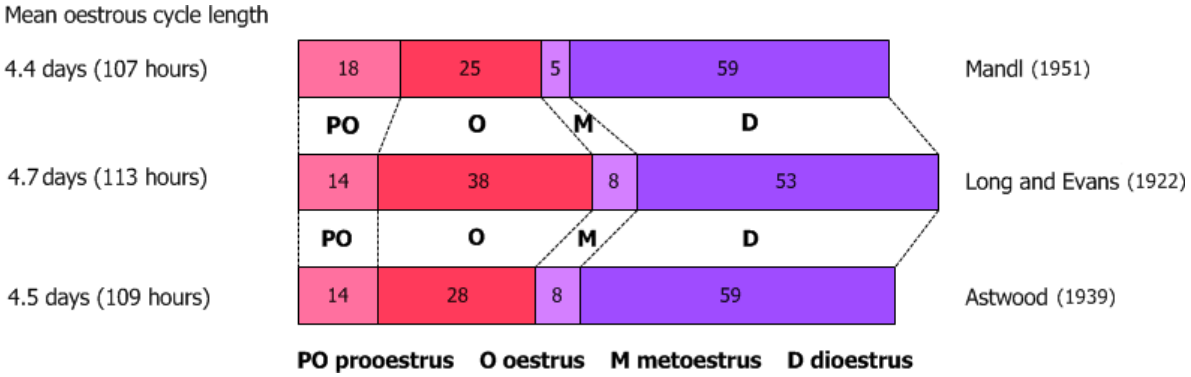
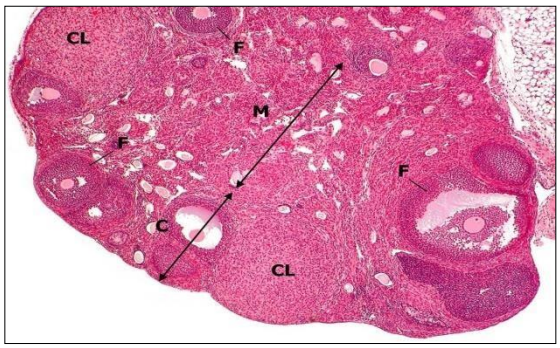


Figure 9– Duration of 4stages of estrous cycle

1. Normal histology of female reproductive tract ovary of rats

The ovaries in rat are grape like structures and vary in size and appearance depending on the various stages of oestrous cycle. The anatomy of the ovaries is shown in figure 10 wherein a single layer of modified peritoneal mesothelium is covered on its surface. The ovarian surface epithelium (OSE) is continuous with the broad ligament mainly mesovarium and supports the ovary. The ovarian surface epithelium can be squamous or cuboidal, columnar or pseudostratified or columnar, this regional variation in OSE morphology accompanies the cyclical changes and happens within the ovarian parenchyma during the oesterous phase (Figures 11 and 12)



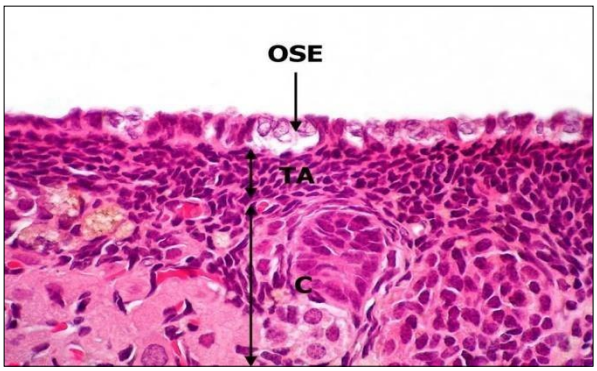


Figure 10– anatomy of the normal rodent rat ovary. C is the cortex which contains numerous follicles, M is the medulla which has lymphatics and blood vessels,follicle is the corpus luteum F is the follicle

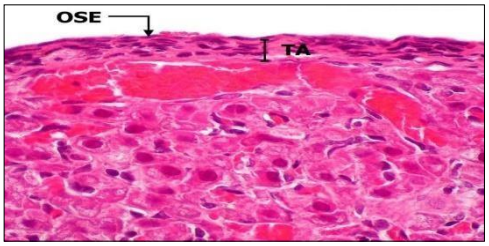


Figure 11– Ovarian surface epithelium (OSE) – columnar type, TA is the tunica albuginea, C is the cortex

Figure 12– The surface epithelium of rodent ovary (OSE) – Simple squamous epithelium (rat, H&E x40).

In figure 10 it is demonstrated that each ovary and oviduct are covered by mesovarium completely in a single compartment called as ovarian bursa (Figure 12). The ovarian bursa in rodents communicates with the peritoneal cavity through a slit like opening

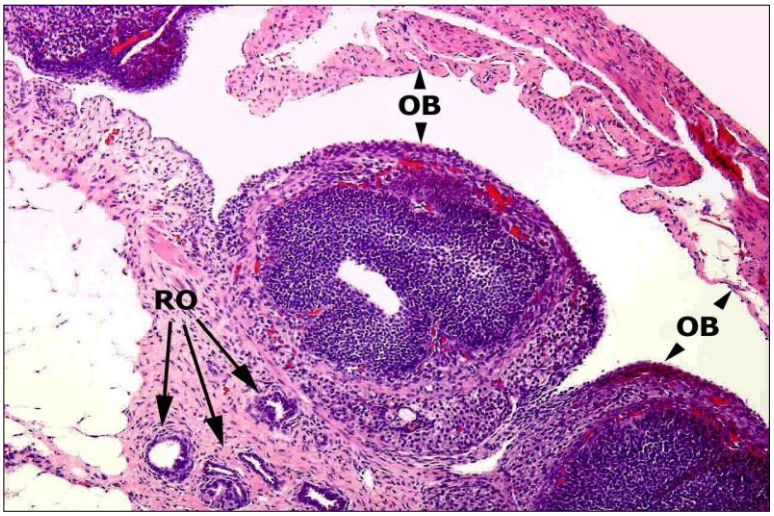


Figure 12 – Ro is the rete ovaries and OB is the Ovarian bursa

Figure 11 shows that ovarian stroma is formed by the body of the ovary and is made up of spindle shaped, fibroblastic cells and collagen fibers along with ground substance.

The dense and fibrous layers covering the stroma of OSE which forms a narrow and distinct zone called as tunica albuginea and the ovarian stroma beneath the tunica layer is further divided into peripheral cortex and central medulla. Although the cortex is not visible in histological sections of ovary.

Figure 12 demonstrates the rete ovariiwhich is observed histologically. this structure

originates from the cells which are mesonephric in origin and they further migrate into the developing gonads during the embryogenesis. in the adult rat, this structure, rete ovarii is made up of several groups of tubules anastomosing with the ovarian stroma and is lined by the cuboidal or columnar epithelium cells

Numerous follicles are present in the sexually matured rats which are present in the cortex suring various stage if development.

The follicular maturation – folliculogenesis is divided into 5 stages –

Primordial follicle – this stage represents the early stage of folliculogeneis. These follicles are formed during early fetal development and they are located within the peripheral cortex beneath the tunica albuginea. these primordial follicles are made up of primary oocytes and is covered by squamous follicular epithelium. This arrangement arrests development of germ cells at the 1st meiotic division. During each oesterous cycle a group of resting primordial follicles starts developing into primary follicles and this phase until the formation of early tertiary follicles.

1) **Primary follicle**- The Squamous follicular cells are surrounded by the primordial follicles which differentiates into single layer of columnar cells forming the primary follicles. (Figure 13 and 14)

Figure 13– Primary and early secondary follicles (rat, H&E x40)., O is the primary oocyte. Z is the zona pellucid,OSE is the ovarian surface epithelium, TF is theca folliculi TA istunica albuginea

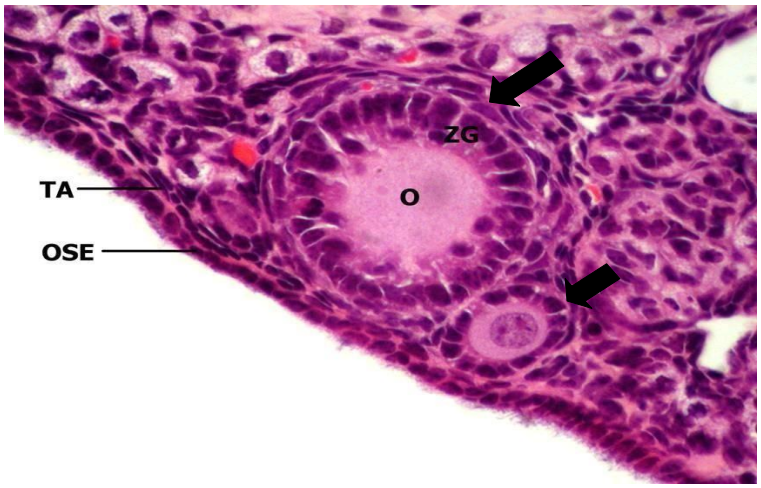


Figure 13– Primary and early secondary follicles (rat, H&E x40)

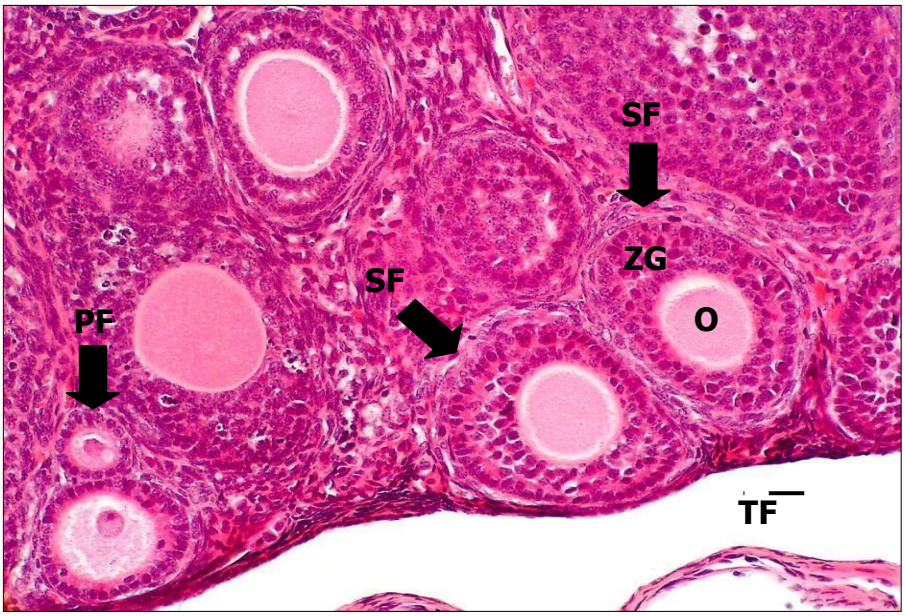


Figure 14– Primary (PF) and secondary (SF) follicles (mouse, H&E x20).

1) **Secondary follicle –** the single layered columnar cells proliferate to form the multilayered zone of granulose cells called as Zona granulosa, surrounding the oocyte. (Figure 13 and 14). Simultaneously the development of thick glycoprotein and proteoglycan coat called as zona pellucida is formed between the oocyte and the zona granulosa. later the stage called as vesicular follicle is formed when the secondary follicle grows and multiple fluid spaces are formed within the zona granulose. The ovarian stroma cells around the developing follicle arranged into concentric layers to form theca folliculi or theca layers. This layer is separated from the xona granulose cells by the basement membrane (Figure 15)

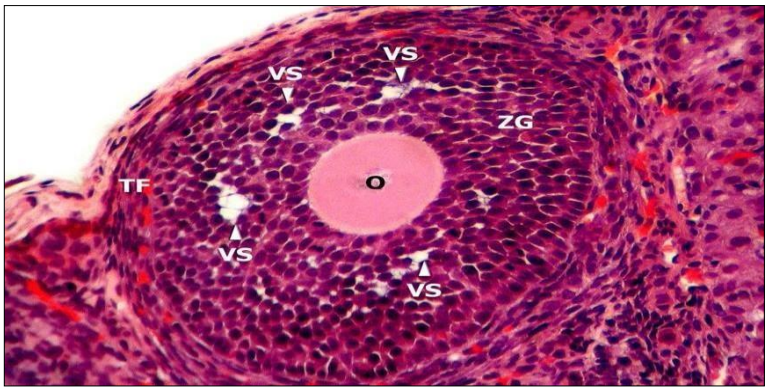


Figure 15– Vesicular follicle (mouse, H&E x20)*. Vesicular spaces (**VS**) are clearly visible within the zonagranulosa (**ZG**).*

Tertiary follicle – the spaces within the zona granulose forms a large central cavity called as follicular antrum. this cavity is filled with fluid called as liquor folliculi and is surrounded by the zona granulosa. In figure 16, the primary oocyte is positioned centrally within the tertiary follicle within the granulose cells and is called as cumulousoophores, which protrudes into the antrum. the granulose cells immediately surrounding the primary oocyte is called as the corona radiate. Figure 16. the tertiary follicles are divided into 2 zones the theca interna and the theca externa. the interna layer is made up of polygonal cells with vacuolated cytoplasm and vesicular nuclei, these cells have steroid producing cells and the main site of synthesis of sex steroid called as androstenedione., while the cells of theca externa are spindle shaped and merge with the ovarian stroma but they do not serve endocrinal function

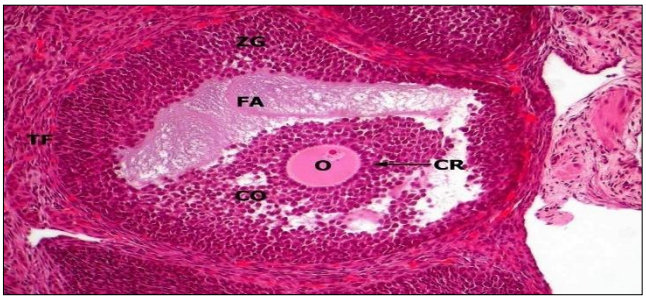


Figure 16 – Tertiary follicle (rat, H&E x20). The large follicular antrum (**FA**) and eccentrically positioned primary oocyte (**O**) characterize this stage.

2) GRAFFIAN FOLLICLE – preovulatory phase – the tertiary follicles enter into preovulatory stage and undergoes various morphological changes. The antrum of the follicle continues to enlarge and suppresses the growth of the surrounding zona granulose. Later the degeneration of the granulose cells of the cumulous oophores occurs and this causes the primary oocyte to detaches from the zona granulose and floats in the follicular antrum freely. The primary oocyte completes the 1st meiotic division prior to ovulation and forms the secondary oocyte. (figure17)

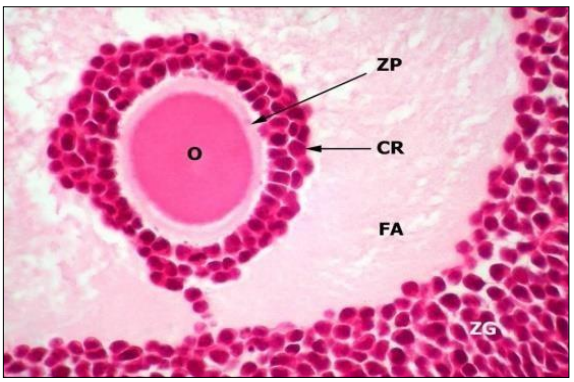


Figure 17– graafian follicle ,O is the primary oocyte, ZP is the zona pellucid, CR is the corona radiate,ZG is zona granulosa

Luteinization – is the process in which the secondary oocyte forces out the graafian oocyte . The other granulosa cells and the theca cells which are remnant outside undergoes hypertrophy and hyperplasia under the influence of LH and prolactin hormones . these two hormones are the main hormones in the rodents helping the luteinization . There is also degeneration of the basement membrane separating the theca interna and zona granulosa,and in flow of the follicles after the ovulation. This results in the mature corpus luteum, which is the large structure filled with eosinophils and it bulges out from the ovarian surface and depending on the location it also comes out from the ovarian corticomedullary junction.

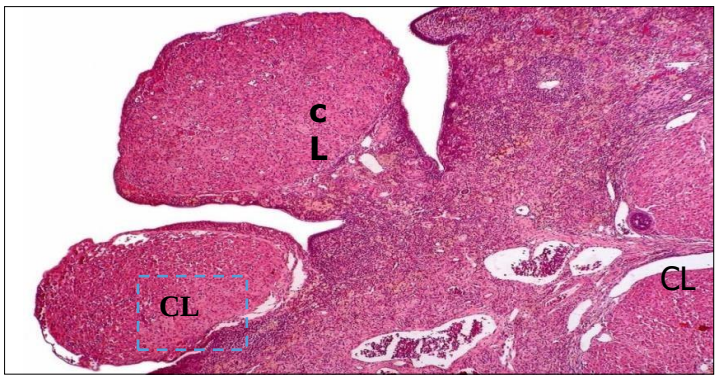


Figure 18– Corpus luteum (rat, H&E x40). The luteal cells (**LC**) comprising the corpus luteum are plump and polygonal; they contain large nuclei and moderate amounts ofeosinophilic cytoplasm. Cytoplasmic vacuoles form within luteal cells as the corpus luteum matures and subsequently degenerates.

Numerous blood vessels (**BV**) arepresent, consistent with this structure's function as a temporary endocrine gland.

Corpora lutea (CL) (rat, H&E x10). *Another pair of corpora luteaare present within the body of the ovary.*

Corpus luteum which matures during the oestrous cycle which is formed before integration of subsequent cycles. At least 3 sets of the corpus luteum are present within the ovaries of normal oestrous cycle rats. The other degenerative corpus lutea shrinks in size progressively and there is increase in number of fibrous tissues and the pigment which is yellowish brown and lipofuscin. The finally formed structure is called as Corpus albicans (white body) in rats this structure undergoes complete regression and no fibrous tissue is remanent within the ovary .

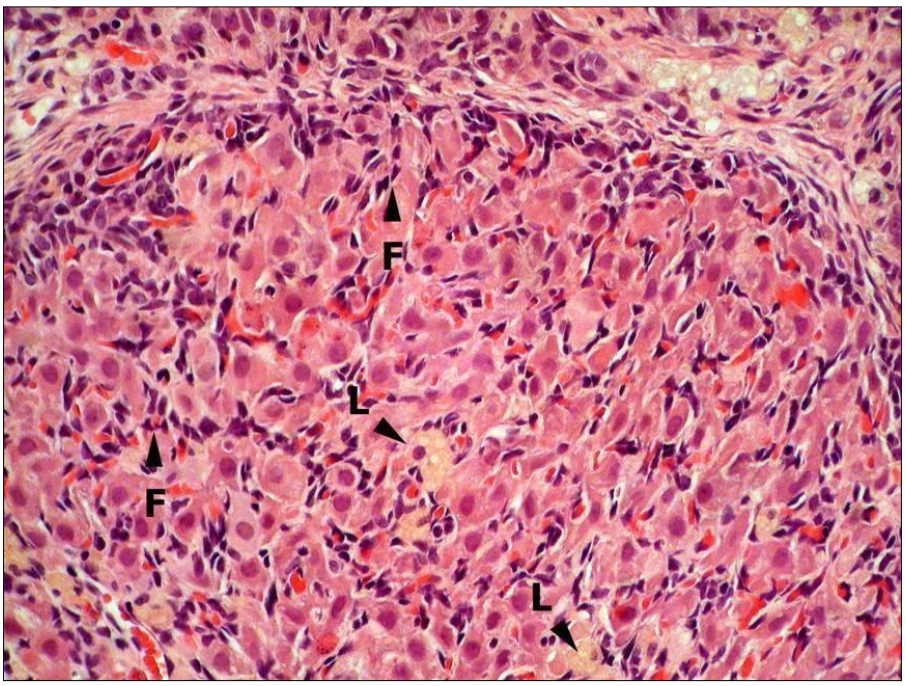


Figure 19-Degenerating corpus luteum (rat, H&E x20). Note the invading fibroblasts (F) and yellow-brown pigment (lipofuscin) (L).

Only few primordial follicles undergofolliculogenesis to form the graafian follicle and undergoes ovulation. The other follicles degenerate or becomes atretic at various stages of follicular maturation .

In rodent rats the degrative tertiary follicles gives rise to interstitial glands within the stroma of the ovary. The glands are made up of theca interna cells and arranged around the degenerative zona pellucid cells . these structures break up into small group of interstitial cells that scatters throughout the medulla

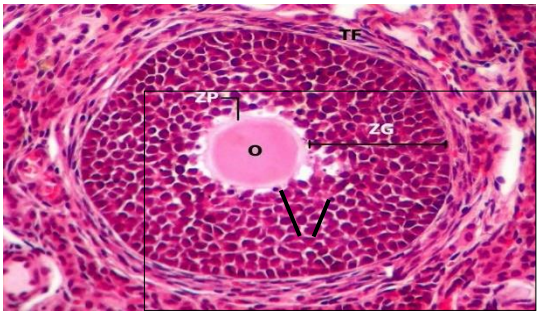


***Figure 20– Atretic secondary follicle (mouse, H&E x20)**. Apoptosis (**A**) is visible in the zonagranulosa (**ZG**) of this degenerating secondary follicle.*

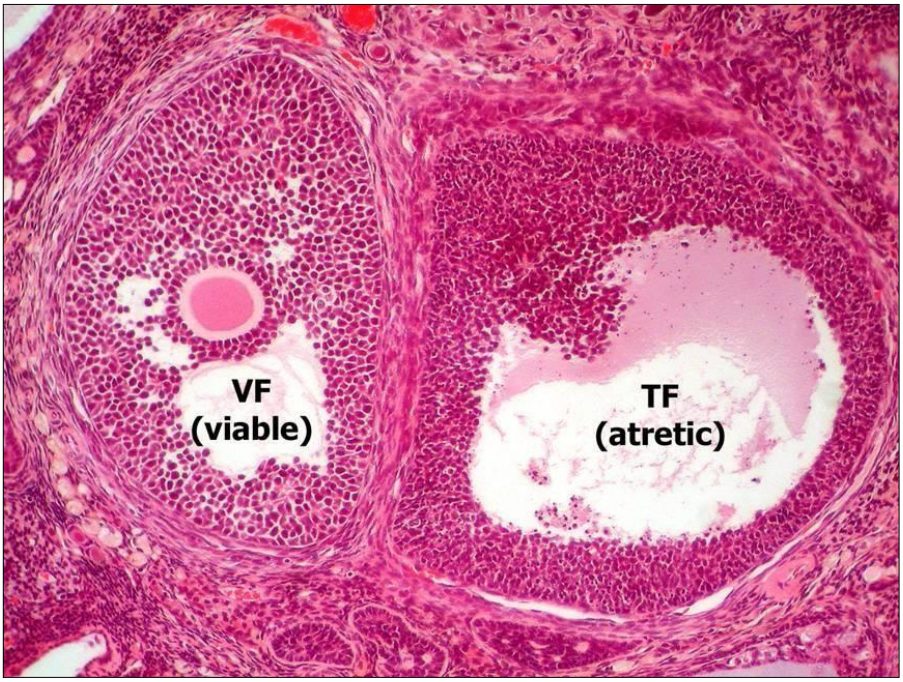


***Figure 21– Degenerate (atretic) tertiary follicle (TF) (rat, H&E x10).** A viable vesicular follicle (**VF**) is also present.*

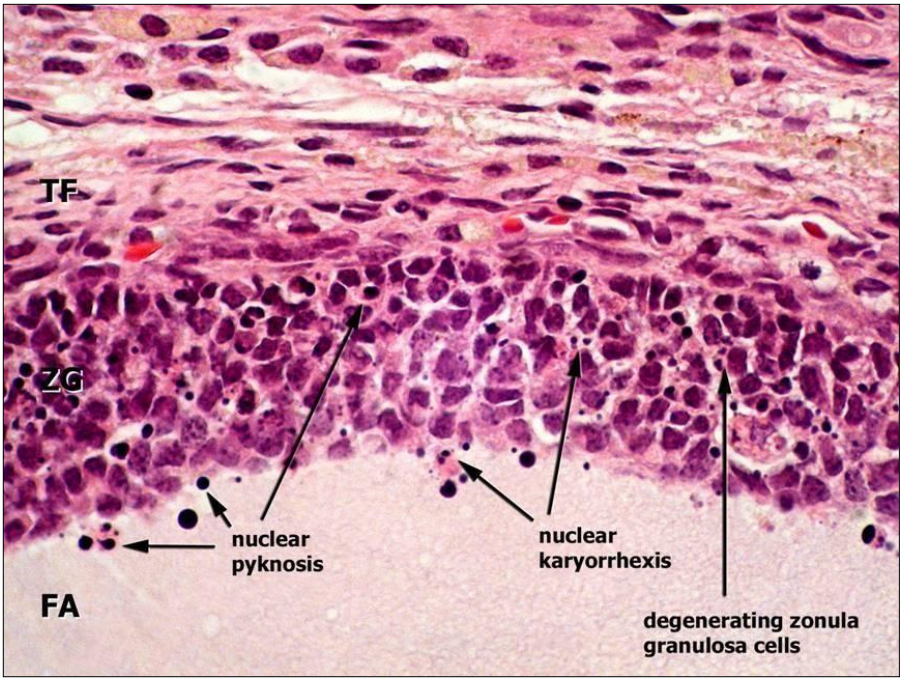


Figure 22– Degenerate (atretic) tertiary follicle (rat, H&E x40). *Pyknotic nuclei and karyorrhectic nuclear debris are scattered throughout the degenerate zonulargranulosa (**ZG**)*

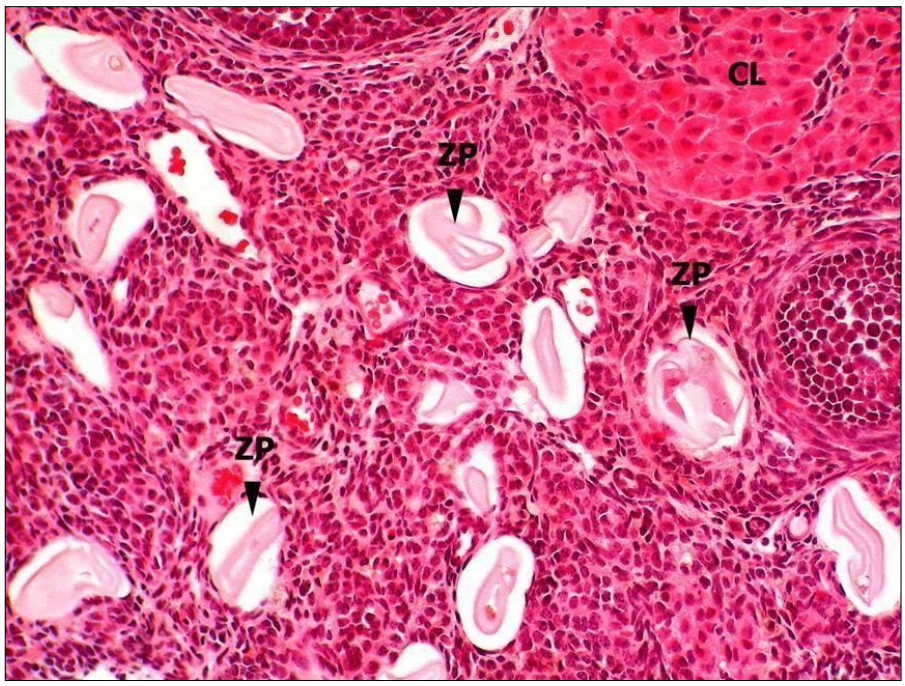


Figure 23– Interstitial glands (mouse, H&E x20). *Theca interna cells surround remnants of degenerate zonaepellucida (**ZP**).*

UTERUS –

The uterus of the rat is comprised of two uterine horns that joins together and opens into vagina through 2 separate cervices.

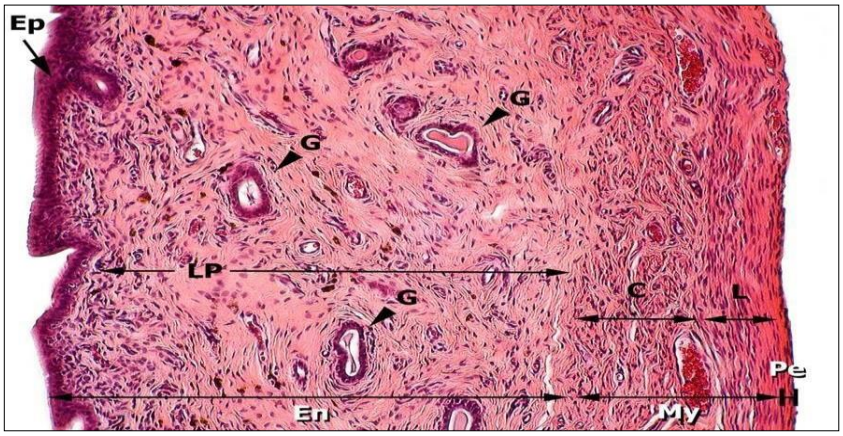


Figure 24– longitudinal section of uterine horn of the rat

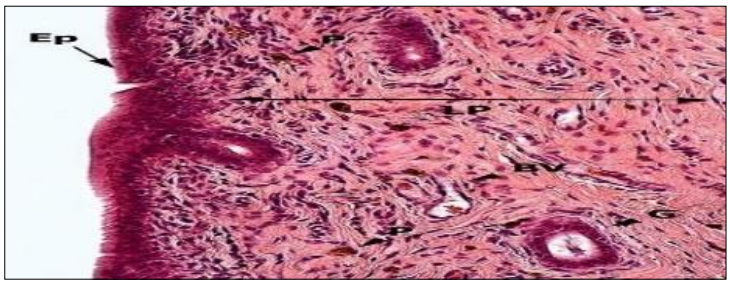


Figure 25– Endometrium (rat, H&E x20). The variation in surface epithelium height is an artefact of sectioning

The endometrium layer is made up of surface columnar epithelium over the thick lamina propria which contains numerous blood vessels and endometrial glands. The myometrium is composed of inner circular and outer longitudinal smooth muscles . the myometrium is covered by the perimetrium layer which is thin connective layer. Many lymphocytes and leucocytes are present within the superficial lamina propria of the endometrium. It is observed that within the lamina propria of the postpartum mature rodents – pigmented stromal cells contain ferritin, hemosiderin and lipofuscin

VAGINA

The vagina consists of the inner mucosa, middle muscularis and outer adventitia layer.

The vaginal mucosa consists of lamina propria which is covered by stratified squamous epithelium. The mucosa undergoes cyclic changes over the course of oestrous cycle. The smooth muscle bundles of the muscularis layer are made up of inner circular and outer longitudinal layers . this layer merges with the adventitia which is a thin connective tissue layer

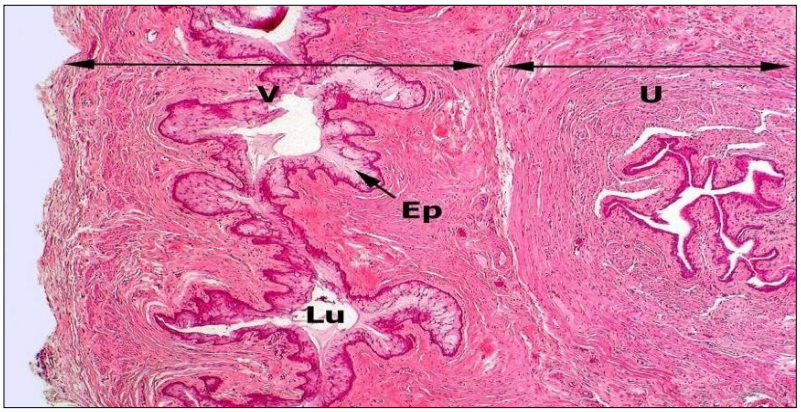


Figure 26– Transverse section through vagina and urethra (rat, H&E x4).

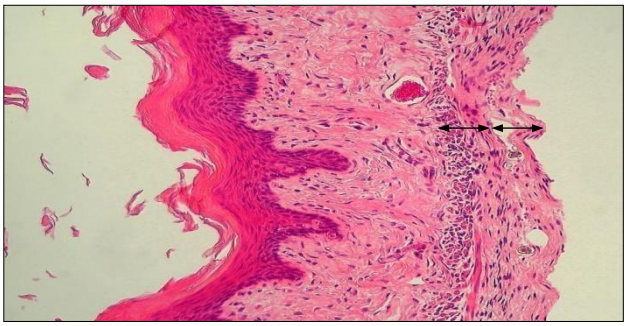


Figure 27– Transverse section LU is the vaginal lumenE is the surface epithelium L is lamina propriathrough vagina (rat, H&E x10).

REVIEW ON INFERTILITY

Definition

Infertility is a condition in which even after having regular unprotected sexual intercourse for more than one year, the couple is unable to conceive.

Classification of infertility

1. Primary infertility: females who never conceived in spite of regular unprotected coitus.
2. Secondary infertility: inability of female to get pregnant subsequently even after having previous successful conceptions.

Among Healthy couples, some females get conceive within one menstrual cycle is defined as fecundability.

Incidence

There are increasing numbers of infertility cases since last decades.[47] The fertility rate is getting deteriorated. According to data mention by WHO which explains there are as many as 60-80 million of couple are infertile in the world. Many couples unable to conceive even though not having any specific causative factor comes under unexplained infertility. With regular unprotected coitus some 80 percent of couples are able to conceive within one year. Remaining couples may get conceive by the end of second year.[48]

Causes	Percentage (%)
Male	30-40% of cases due to male factor includes sperm morphological abnormalities, motility, sperm count.
Female -Ovulatory	30- 40% of cases due to anovulation, luteal phases difficult.
Tubal	25-35% of cases due to tubal factors includes tubal scarring, defect in ciliary movements, tubal adhesion.
Uterine and cervical	5-10% of cases due to cervical causes uterine factors.
Unexplained	Remaining 10% includes under unexplained infertility.

Table No 1 : Causes of Infertility with percentage [49]

Causative Factors[50]

Male Causes - There are many male causative factors responsible for infertility.

Abnormal sperm production

Due to undescended testicles, genetic defects, health problems such as diabetes or infections such as chlamydia, gonorrhea, mumps or HIV. Enlarged veins in the testisvaricocele also can affect the quality of sperm.

1. **Blockage in the ejaculatory ducts:** Congenital absence, infections and any trauma may cause blockage in the ejaculatory ducts surgical injuries may cause blockage in epididymis, vasa or ejaculatory ducts. Infections cause by gonorrhea or tuberculosis also cause lesions in vas deferens. The blockage in ejaculatory ducts interferes with proper passage of semen.
2. **Problems to deposit sperm in the vagina:** Premature ejaculation, abnormalities of penis such as hypospadias is some drugs which influence ejaculation are B-blockers, phenothiazines, thiazide etc. may affect ejaculation. Phimosis causes improper deposition of sperm in the vagina. Impotence is a condition in which there is failure to ejaculate sperm.
3. **Problems in the sperm quality:** Sperm count, fructose and prostaglandins are constituents of seminal fluid. Any variations in semen quantity and quality cause infertility.
4. **Endocrine dysfunction:**Testosterone is the hormone which stimulates sperm production. Any deficiencies in testosterone causes impair sperm production. Diabetes mellitus, thyrotoxicosis or frolic syndrome is other causes which also hampers sperm production.
5. **Genetic factors**: Normal male karyotype is essential for proper sperm production. In Klinefelter syndrome which have two X chromosome and one Y chromosome leads to low sperm count, low sperm count due to abnormal development of testis.
6. **Excess alcohol consumption**: The man who consumes alcohol impairs body metabolism. Interferes with sperm quality and quantity. Most of them have low sperm count known as oligospermia it will decreases the fertility.
7. **Drugs and chemicals:**Many drugs used by man for various diseases such as steroids, methotrexate,sulfasalazine,nifedipine , nicotinic decreases the ability of the sperm to fertilize the ovum. These drugs reduce sperm count and reduced sperm motility.
8. **Chemotherapy:** The drugs used in chemotherapy have many side effects. Itseffectssperm count. Decreases sperm count.

Female Causes

Ovulation dysfunction (30-40%)	Oligoovulation , Anovulation ,Corpus luteum deficiency
Tubal abnormalities (25-35%)	Tubal block may be due to infection, pelvic adhesions etc.
Uterine abnormalities (10%)	Thin endometrium, endometritis , uterine fibroids, Synechiae, congenital abnormalities .
Cervical factors (5%)	Cervicitis, cervical polyps, cervical erosion, cervical malignancy.
Vaginal Factors	Vaginal atresia, vaginal septum,Narrowintroits,Vaginitis and purulent discharge

Table 2 – Cause of infertility in female

Functional disarrangement of ovary is the major cause for infertility.

WHO classifies Ovarian dysfunction into seven main groups (WHO 1976) [51]

- Group 1: Complete absence of function of Hypothalamic –Pituitary axis.
- Group 2: partial dysfunctionof Hypothalamic –pituitary axis.
- Group 3: Ovarian failure – loss of follicular activity.
- Group 4: Congenital or Acquired female reproductive tract abnormalities.
- Group 5: Pituitary tumors causes'hyperprolactinemia .it is coined as lesion in the Hypothalamus-pituitary region due to space occupancy
- Group 6: Hyperprolactinemia without any tumors is coined as a lesion in the Hypothalamus-pituitary region without any space occupancy.
- Group 7: Amenorrhea with an any tumors or mass is coined lesion in the Hypothalamus-pituitary region with space occupancy.

Anovulation

Anovulation is a common cause for female infertility in today's generation. The ovarian function is under the influence of gonadotrophins which is depending on the pulsatile secretion of GNRH from the hypothalamus, hence ovarian dysfunction is linked with disturbed hypothalamo-putuitary-ovarian axis. In anovulatory condition though their serum FSH concentration is normal, menstruation will be irregular and excessive as endometrium is proliferated under influence of estrogen and there is no progesterone synthesis. The endometrium is

shredded by sudden withdrawal of estrogen and there is excessive and irregular shedding of endometrium. But their serum FSH concentration will be normal.[52]

Anovulation means absence of ovulation .It is characterized as menstrual bleeding without preceding ovulation and followed by corpus luteum formation.

Conditions essential for ovulation to occurs normally are,

- Hypothalamic pituitary ovarian axis must be intact with pulsatile secretion of GnRH.
- Ovarian hormones must have good response at their respective target organs.
- Positive and Negative feedback signals to be properly active .
- Any abnormalities in above factors results in anovulation.

Types of anovulation

Primary Anovulation: If a woman has never ovulated it is said to be primary anovulation

Secondary Anovulation: Suspension of ovulation secondary to some other illness is considered as secondary anovulation.

Pathophysiology of Anovulation

Follicular growth is independent till it attains the size of 2-5 mm. after that follicle are recruited by follicle stimulating hormone. During menstrual phase and even prior to it, due to absence of negative feedback of estrogen ,progesterone and inhibin ,anterior pituitary secretes FSH. FSH is responsible for follicular growth, helps in maintaining follicular microenvironment estrogen dominant rather than androgen, which is essential for continuous follicular growth and development into dominant follicle. Further FSH induces receptors for LH activity in granulosa cells which is needed for ovulation and luteinization process.

The factors responsible for ovulation are LH surge. Before this there is estradiol surge which initiates ovulation. LH surge is essential for triggering of ovulation and follicular rupture about 36 hours after the surge. Other functions are disruption of cumulus oocyte complex, induction of the resumption of oocyte meiotic maturation and luteinisation of granulosa cells.

Following ovulation there is formation of the corpus luteum, increasing concentration of progesterone slow down the frequency of the LH pulses. Luteal phase is constant in each menstrual cycle i.e., 14 days, during which FSH and LH

levels are low. After luteal phase, corpus luteum gets degenerated, progesterone levels fall. Again, FSH increases to recruit follicles for next menstrual cycle. The coordination between the follicle and hypothalamic pituitary ovarian axis and all gonadotropins those are FSH,LH, and gonadal steroids estrogen inhibin is responsible for ovulation.

This recycling mechanism is regulated by substance functioning as classic hormones (FSH, LH, estradiol and inhibin) transmitting messages between the ovary and the hypothalamic-pituitary axis and autocrine/paracrine factors, which co-ordinate sequential activities within the follicle designated to ovulate. Due to improper response to stimulus, improper function of IGF-2, inhibin and activin causes dysfunction of follicular receptor activity within the ovary.

Additional Factors Responsible for Causing Infertility

Age and Infertility

Fertility period of woman is determined by woman's age. Germ cells undergo repeated mitotic division in the fetal ovary itself. The oogonia present in female fetus around 16-20 weeks measures about 6-7 million. There number declines as the fetal grows further. Till it reaches puberty the number of oogonia presents about 3 lacs only. During reproductive age, the number further declines. As age of the woman increasing the quality of oocytes decreases.

Serum levels of FSH and LH get alter according to age of woman. Before attaining menopause, there is normal levels of LH hormone. Prior to menopause serum levels of FSH is more. The length of follicular phase and luteal phase will vary. The follicular phase will be shorter and luteal phase will be normal.

As the age advances the quality of oocyte will be perished. There may be some chromosomal abnormalities occur in fetus such as trisomy 21, downs syndrome, aneuploidy , abortions etc.in elderly gravida. During active reproductive period, healthy and good quality oocyte are ovulated. As age advances there will be few follicles left in the ovary and oocyte have abnormal chromosomes [19.]

Smoking and Alcohol[53]: The risk of fertility problems increases with number of cigarettes smoking. Smoking causes untoward effect on health it hampers proper growth of follicle; it reduces number of follicles. It has negative impact on health of woman and disrupts the functions of hypothalamic ovarian axis.

Stress and Emotion: Due to continuous anxiety and depression due to stressful life, pressure from family increases level of stress hormones in the body. It reducing fertility rate among woman. A study revealed that due to increased stress about 25-60% of females have altered mental faculty compared to fertile couple.

Faulty Diet Habits: Increased consumption of junk food, processed food having many preservatives, coloringagent's causes dysfunction of hypothalamic pituitary ovarian axis responsible for anovulation.

Exposure to Chemicals: There are certain chemicals which interfere with fertility of man and woman, such as marijuana hampers the release of GnRH and cocaine inhibits spermatogenesis. Caffeine inhibits fertilization of sperm and ovum and delays conception. Increased use of fertilizers and pesticides and exposure to them causes infertility.

Infections: Vaginal ph. maintains acidic ph. prevents the ascending infection. But due to loss of vaginal defense the ascending infection affects the epithelial lining of vagina and cervical cavity. It reaches further to fallopian tube and destroys the ciliary lining of the fallopian tube. The infection further causes endometritis, salpingitis and cervicitis. Infection interferes with mobility of sperm. Scaring in the tube and uterine cavity causes inhibits transportation of morula and implantation in endometrial cavity. Chlamydia, trichomoniasis and bacterial vaginosis interferes with fertility of woman.[24]

Obesity and Infertility: Obesity affects fertility rate in woman. Increased weight in woman even though ovulate normally and attain conception but spontaneous conception is affected by increased weight gain , as obesity disturbs body metabolism. Excessive weight gain with increased body mass index than normal and woman with undernutrition with loss of weight both will hamper ovarian function which is explained by Imani et al.

As the age advances the woman gain more weight usually and increased weight hampers fertility rate. WasiuEniola et al Increased adipose tissue has more aromatization which increases peripheral secretion of estrogen. Increased serum levels of serum estrogen and obesity affects fertility rate. [26]

Rapid Weight Loss:Undernutrition and low-calorie diet cause malnourishment in woman. It dysregulates menstrual cycle and ovulation. It can be regulating by proper intake of nutritious and required calorie diet.

Genetic Considerations[54]: Secondary infertility is also due to systemic or syndromic genetic defects including developmental endocrine and metabolic defects. Genetic factor also has prime role in infertility. Increased prevalence of anovulation in family and increased cases of polycystic ovarian syndrome had hereditary history due to mutation in some genes and may be due to X linked dominant transmission.

Hypothalamic Cause: Pituitary is said to be as master gland in human body. Hypothalamus secretes releasing hormone which stimulates anterior pituitary gland. FSH and LH are gonadotropins which stimulate follicular growth in the ovary which further synthesizes gonadal steroids maintains menstrual cycle and ovulation . Any alteration in this process causes anovulation. Ovarian failure is responsible for 20% of infertility cases in female.

Duration of sexual life in months	**% Chance of conception**
3	57
6	72
12	85
24	93

Table 3 - Time required for fertility[55]

Conception rate is directly proportion to duration of sexual life in months. More the duration of sexual life more the chances of conception.

Investigations[56]

Both the couples should be investigated after one year of trying with unprotected regular coitus to know the underlying pathology which are preventing them from conceiving. If the couple are of more than 35 years they should be investigated early.

Objectives of investigation

Investigation plays a pivotal role in the workup for infertility. The results must be assessed carefully as theses assess dynamic function. Investigation should be done in infertile couple who are unable to concept in spite of regular unprotected coitus after one year. Investigation should be done even earlier in the couple who are more than 35 years of age.

Objectives of investigation of infertile couple are as follows

- To identify exact causative factors responsible for infertility in male and female patients.
- To administer appropriate treatment protocol to correct infertility.
- To enhance psychological assurance and strength in infertile couple.

Investigations for Male Factors

1. Semen analysis : to analyze sperm count , ph., viscosity,
2. Scrotal scanning: to identify lump in the scrotum or testicles or a tumor or filled with fluid.
3. Testicular biopsy: to identify scrotum, testicles and blood vessels.
4. Hormonal assay : to identify serum levels of FSH, LH, AMH and testosterone

Investigations for Femalefactors

Tubal Factors: Theanatomical patency of the tubes is assessed by,

- **Insufflation Test (Rubin'stest)**

Principle: The principle involved is the fact that the cervical canal is in continuity with the peritoneal cavity through the tubes. When CO2 is pushed into cervical canal with high pressure there will be entry if air or CO2 into peritoneal cavity as well. This gives evidence of tubal patency. It should be done 2 days after stoppage of menstrual bleeding as in postmenstrual phase It is contraindicated in cases of presence of pelvic infection.The tubal patency is confirmed by following inferences: (1) there will be fall in the pressure gradient when raised beyond 120 mm Hg, (2) on auscultating on either iliac fossa a hissing sound is appreciable and (3) due to irritation of the diaphragm by the air patient experiences the shoulder pain.

- **Hysterosalpingography(Hsg)**

Instead of air or CO2, dye is instilled transcervical. Principle involved is same as that of insufflation test.

It has got distinct advantages over insufflation test as it can precisely detect the side and site of block in the tube. It can also reveal any abnormality in the uterus caused due to congenital or acquired reasons like synechiae, fibroid and others.

- **Laparoscopy andchromopertubation**

After the evaluation of male factor and ovulatory functions are found to be correct the gold standard method employed to evaluate the tubal factors for infertility.

Diagnostic

- Age > 35years
- Abnormal

1. Failure to conceive after reasonable period (6 months) with normal
2. Women with comorbid pelvic pathology (PID,endometriosis)
3. Unexplainedinfertility

- **Operative**

1. Ovarian drilling
2. Reconstructive tubal surgery
3. Adhesiolysis

- Fulguration of endometriotic implants
- GIFT and ZIFT procedures

It may be done in the proliferative phase mostly performed during secretory phase as recent corpus luteum may be visualized and endometrial biopsy can also be taken at the same time

Sonohysterosalpingography

Normal saline is pushed inside the uterine cavity using a pediatric Foley catheter. To prevent fluid, leak the catheter balloon is inflated at the level of the cervix. Ultrasonography of the uterus and fallopian tubes are done. Through Ultrasound we can follow the fluid through the tubes up to the peritoneal cavity and in the pouch of Douglas.It can detect uterine malformations, synechiae, or polyps (superior to HSG). Tubal pathology, tubal blockages could be detected through this.

1. **Falloposcopy**: The entire length of tubal lumen is studied with the help of a fine and flexible fiberoptic device. Using a hysteroscope it is performed through the uterine cavity, tubal ostia, mucosal pattern, intratubal polyps, or debris can be visualized directly

2. **Salpingoscopy**:By introducing a rigid endoscope through the fimbrial end of the tube the tubal lumen is studied, performed through the operating channel of a laparoscope.

Uterine Factors:Uterine factors commonly associated with subfertility are submucous fibroids, congenitalmalformations and intrauterine adhesions (Asherman's syndrome). They are likely to cause recurrent pregnancy loss rather than primary infertility. Ultrasonography, HSG, hysteroscopy, and laparoscopy are needed for the evaluation of uterine factor for subfertility.

Cervical Factor:Proliferative phase permits the entry of sperm and secretory phase hinders sperm penetration. Abnormality of cervical factor can be diagnosed by,

- **Postcoital test:** It is done to assess the cervical mucus quality and to know if the sperm can survive in that environment. Due to increased estrogen level near ovulation the cervical mucus will be thin and watery.

Procedure-2-12 hours after intercourse in the morning collect the endocervical mucus and examine under microscope.

A Result- 20 motile sperm indicates normal, less or absent motile sperms- need further evaluation.

Post coital Spinbarkeit test-This test is done on expected date of ovulation. Small piece of pH paper should be kept in contact with cervical mucus and lifted vertically.

Result: Mucus should thread for about 6 cm. pH should be more than 6

Diagnosis of Ovulation

Various methods used to detect ovulation are grouped mainly as follows

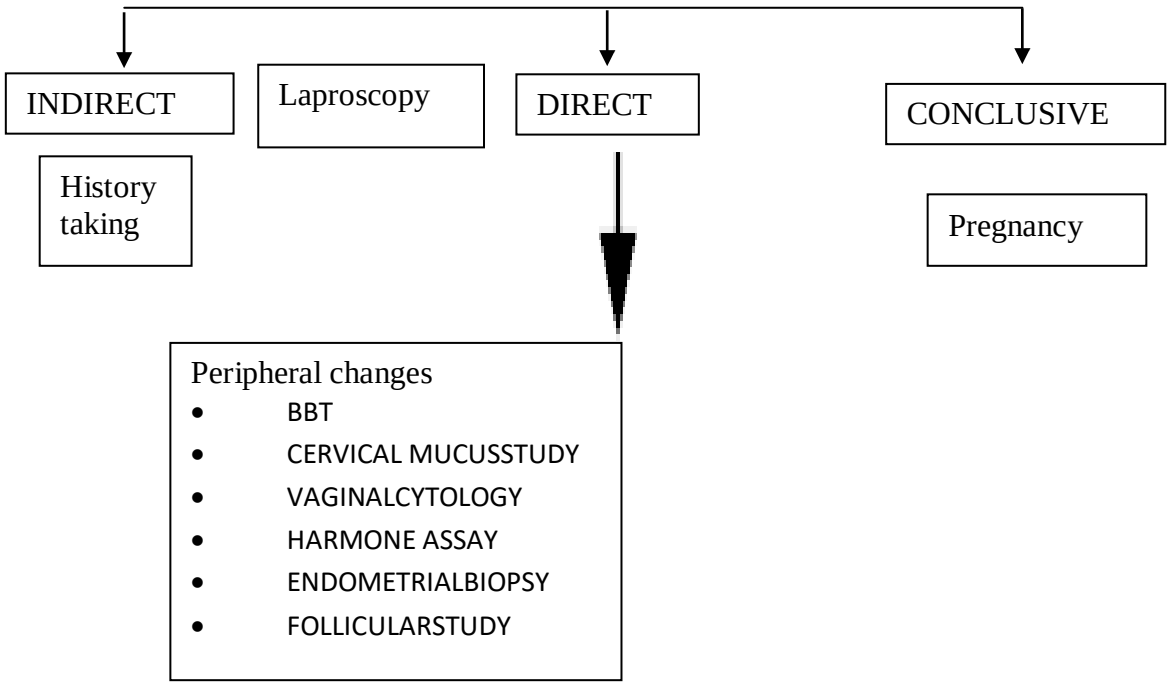


Figure 28- Diagnosis of ovulation

Indirect Method of Diagnosing Ovulation

Menstrual History-

Features of strong evidences of ovulation are,

- Woman aging between 20-35 years with regular menstruation and normal bleeding.
- Excessive mucoid vaginal discharge (Mittelschmerz syndrome) may be evident sometimes woman also experiences spotting or pain in the midcycle.
- Premenstrual syndrome or primary dysmenorrhea.

Evaluation of Peripheral or Endorgan Changes

Estrogen and progesterone are the hormones responsible for follicular changes ,hence by considering the peripheral and end organ changes indirect evidences of ovulation can be withdrawn.

Basal body temperature(BBT) : There is sudden raise and decrease in the temperature in middle of the ovulatory cycle which is termed as "Biphasic pattern". There will be no rise of temperature in anovulatory cycle in any of the day throughout the cycle.The rise of temperature is due to rise in progesterone output leading to ovulation. Progesterone hormone is associated with body thermal regulation and is said to be thermogenic. The prime reason for the rise in temperature is the increase in the production and secretion of norepinephrine which is also considered to be thermogenic.

Procedures: Before rising out of bed patient is instructed to take her oral temperature daily in the morning and it should be recorded in a special chart containing the day and temperature. For better evaluation of frequency of coital history, the days when intercourse takes place should also be noted on the chart.

The body temperature which is maintained throughout the first half of the cycle is raised to 0.5° to 1°F (0.2°–0.5°C) after ovulation. The level of rise in body temperature sustains throughout the second half of the cycle and suddenly falls about 2 days prior to the next period–hence this pattern of rise and fall of temperature is called "biphasic pattern". Before the rise of temperature there may be a drop in the temperature to about 0.5°F and this coincides with either LH surge or ovulation. The considerable rise actually occurs about 2 days after the LH rises to peak and with a peripheral level of progesterone greater than 5ng/ml.

By proper maintenance of BBT chart during the investigation procedure is helpful in

determining the ovulation and also provides specific timings for various investigation such as post-coital test, endometrial biopsy, cervical mucus or vaginal cytology study for ovulation.

Limitations of BBT

- BBT reveals ovulation retrospectively.
- Unable to predict the precise time for ovulation.
- Rarely, ovulation which has been observed through BBT is monophasic.

It should not be continued for more than 3–4 months for investigation purpose.

Cervical mucusstudy: Due to the effect of estrogen and progesterone there occur many changes in physiochemical properties of cervical mucus.

The absence or disappearance of fern pattern beyond 22nd day of the cycle, which was present previously in the midcycle is confirmatory of ovulation. The presence of fern pattern even beyond 22nd day of menstrual cycle suggests anovulation.Sodiumchloride crystals are dissolved due to progesterone. The presence of stretchability (spinbarkeit) which was present at the time of midcycle islost.

Vaginalcytology: Due to the effect of progesterone hormone, there will be shifting of the maturation index to the left side from midcycle to mid second half of the cycle. If ovulation occurs a single smear on 25 or 26^{th} day of the cycle reveals features of effect of progesterone.

Hormone estimation: Through assessment of levels of serum gonadotropin and steroid hormones before,after and at the time of ovulation reveals the indirect method of ovulation.

Serum progesterone: Serum progesterone is to be estimated on day 8 and 21 of a 28 days regular cycle. With increase in value from less than 1ng/ ml to greater than 6 ng/ml suggests ovulation.

Serum LH: LH surge can be estimated by evaluating the levels of serum LH at the mid cycle. After the rise or beginning of the serum LH levels for about 34-36 hours follows the ovulation It coincides about 10–12 hours after the LH peak.

Serum estradiol: About 24-36 hours before ovulation and prior to LH surge the serum estradiol reaches the Peak estimation of serum LH and estradiol is used for in vitro fertilization.

Urinary LH: To detect midcycle LH surge LH kits are available. Within 14–26 hours after the detection of urine LH surge ovulation occurs and almost always within 48 hours. (Usually done on a daily basis. It is started 2–3 days before the expected LH surge depending upon the cycle length).

Endometrial biopsy: Endometrial tissue samples can be taken to detect ovulation; this is done as an outpatient procedure using instruments such as Sharman curette or Pipelle endometrial sampler. Dilatation and curettage are, however reserved in cases where bulk endometrial study is required as in endometrial tuberculosis.

On 21^{st}–23^{rd} day of the cycle the endometrial biopsy is carried out. During the cycle to prevent accidental conception barrier method of contraceptive is prescribed , it is done within 24 hours of the period if the cycle is irregular. By the evidences of secretory activity of the endometrial glands the functional integrity of the corpus luteum as well as ovulation detection can be done in midcycle.

Follicular study-Sonography: Continuous series of Transvaginal sonography just prior to ovulation and comparing them reveals the precise increment in measurement of Graafian follicle.A fully developed follicle measures about 18-20 mm .The collapsed follicle and fluid in the pouchof Douglas are suggestive of ovulation.

Direct Method of Diagosing Ovulation

Laparoscopy: Laparoscopic visualization of corpus luteum or detection of the ovum from the aspirated peritoneal fluid that is taken from the pouch of Douglas are the direct evidences of ovulation.

Conclusive: Without ovulation there is no pregnancy and hence this is the definite evidence of ovulation.

Luteal Phase Defect (LPD)

Diagnosis of LPD is done by following ways:

- BBT chart—(a) There will be slow rise in body temperature taking 4–5 days following the fall in the midcycle. (b) Rise of temperature sustains only less than 10days.
- Endometrial biopsy—Biopsy done on 25–27th day of the period reveals the state of endometrium at least 3 days out of phase. This is called lag phase endometriumthis is not conclusive as it must be proved in more than one cycle.
- Serum progesterone following ovulation is less than 10 ng/ml when estimated on 8^{th} day

Luteinized Unruptured Follicle (LUF) : Luteinized unruptured follicle (LUF) syndrome refers to an infertile woman with regular menses having presumptive evidences of ovulation without release of the ovum from the follicle also referred to as trapped ovum .formation of corpus luteum and its stigma are absent here ,these are the specific indicators of ovulation which are absent in case of LUF. It is often associated with pelvic endometriosis.

Diagnosis: In the early luteal phase the biological effects of progesterone are as follows,

1. Sonography: After 36 hours following LH peak the presence of echo-free dominant follicle
2. Laparoscopy: Absence of stigma of ovulation.
3. Ovarian biopsy: The presence of ovum in mid structure of corpus luteum is conclusive

MANAGEMENT OFINFERTILITY[57]

In general, the first step involves identification of cause and contributing factors then, treatment is amed at correcting them

The role of physician in management of infertility

One should do workouts of the infertility case with these goals.

- Proper evaluation and correction of causes.
- To provide proper information to couple
- To provide emotional support

Counseling the couple: The infertility management includes- The patient assessment , counseling and management[26].

The steps start with,

- History
- Inspection
- Interrogation
- Clinical examination – general and gynecological
- Investigation

History

- Detail history should be taken about age of couple, their occupation, diet, lifestyle, habits, and marital life. For infertility to cause these factors play importantrole.
- Previous and present medical treatment history about sexually transmitted disease, diabetes mellitus, thyroid dysfunction, hypertension, mental disorders etc must betaken.
- Past surgical history especially any abdominal or pelvic surgery is needed to rule out pelvicadhesions.
- Menstrual history for interval, duration and amount of bleeding is to be taken along withcolour of menstrual blood, pain if any, consistence of menstrual blood and odour also.
- If pelvic endometriosis is there it female may have premenstrual dysmenorrhoea and painful coitus indicates any pelvicpathology.
- History on milk discharge from the breasts to rule out hyperprolactenemia which is present in amenorrhoea isnecessary.
- History on anosmia as in Kallamans syndrome should betaken.
- Tuberculosis of any part of the body may indicate the genital tuberculosis; hencethe related history should be taken.
- History about coital frequency also should betaken.

Interrogation: One should have patience to talking to infertile couple. Female with secondary infertility needs to be asked about obstetrical details.

Enquiry should be made related to number of previous conceptions, the gap between two pregnancies, number of live births, about any complications, any ill health. If the history reveals any previous infectious conditions that may leads to uterine adhesions or even tubal block.

Contraception history also important as using IUCD may cause PID

Clinical Examination

- Should observe the nutritional state of the female as increased and decreased body weight has direct role in causing infertility.
- Look for development of secondary sexual characters.
- Observe for acne, excessive hair growth on face, chest, abdomen, blackish discoloration of nape of neck suggesting acanthosis nigricans which are

present in polycystic ovarian syndrome.

- Systemic examination to diagnose hypertension, heart disease, chronic renal lesion, endocrinopathies
- Careful systemic examination can reveal hypertensive disorders, heartdisease, renal dysfunction, endocrinopathies.
- Gynecological examination should be done carefully.
    - Inspection: external genitalia: we can exclude imperforated hymen, narrow introitus, and anyabnormal growths.
    - Per speculum examination can reveal cervical lesions like cervicitis, cervical erosion, cervical polyps, and elongated cervix.
    - Per vaginal examine reveals size of uterus, position of uterus. Also, mobility of uterus. By palpating the fornixes, we can exclude adnexal masses. Tenderness presence hints about infection. Presence of nodules in the Pouch of Douglas.

Couple Instructions

Assurance: The infertile couple remain psychologically disturbed right from the beginning, more so as the investigation progresses. The couple in such cases should be sensitively handled to minimize psychological upset.When minor defects are detected in both the husband and the wife, each of which individually cannot cause infertility but in combination, they significantly decrease the fertility potential. As such, the faults should be simultaneously treated and not one after the other**.** Even when a gross abnormality is detected and the prospect of pregnancy is bleak, an optimistic discussion is worth rewarding.

Body weight: Overweight or underweight of any partner should be treated, to obtain an optimum weight. Body mass index of 20–24 is optimum.

Smoking and alcohol: Excess smoking or alcohol consumption has to be avoided.

Coital problems: The coital problems should be carefully evaluated by intelligent interrogation. Advice to have intercourse during the midcycle too often gives the result even prior to investigation. Using LH test kit, one can detect LH surge in urine by getting a deep blue color of dipstick. The test should be performed everyday between day 12 and day 16 of a regular cycle. Timed intercourse over 24–36 hours after the color change reasonably succeeds in conception. Minor psychosexual problems should be dealt with accordingly.

For convenience, the treatment modalities in female infertility are grouped as follows according to the disorders identified:

- Ovulatory.
- Cervical.
- Associated disorders like endometriosis
- Infections or endocrinopathy.
- Tubal.
- Immunological.
- Unexplained infertility.
- Uterovaginal canal.
- Assisted reproductive technology(ART).

Estimation of the Time and Frequency of Ovulation

After the basic work-up has been done, it may be better, even in case of regularly menstruating women, to look for presumptive evidence of ovulation or luteinisation. The method for assessment which has been over emphasized in recent years is the daily temperature record. This method is advantageous because it indicates the approximate time of ovulation, and thereby the time when coitus is most likely to be fruitful. Ovulation pain (Mittelschmerz) is also an indication of ovulation.

Ovulation Induction

Ovarian dysfunction is the most common indication for the ovulation induction. These agents can also be used in ovulatory women to increase the likelihood of pregnancy in couples with other causes of infertility or unexplained infertility. Use of these medications to promote follicular development and prompt ovulation is called superovulation or ovulation enhancement. If these agents are administered solely to stimulate follicles, and egg harvesting is completed by ART, then the term controlled ovarian hyperstimulation is used. In contrast, we prefer the term ovulation induction to describe treatment with medications to stimulate normal ovulation in women with ovarian dysfunction. Frequent causes of ovarian dysfunction include PCOS and diminished ovarian reserve. Less often, central (hypothalamic or pituitary) disorders or thyroid dysfunction can result in infertility.

Rarely, ovarian tumors or adrenal abnormalities lead to abnormal ovarian function. Treatment of ovarian dysfunction should be based on the identified cause as well as the results of any prior attempted therapy.

Management Of Anovulation with Specific Drugs

The common drug used for induction of ovulation is CLOMIPHENE CITRATE. The mode of Action of clomiphene is to suppresses the estrogenic feedback on hypothalamus and regulates the proper secretion of gonadotropins and useful in anovulatory and luteal phase dysfunction. The estrogen is a gonadal steroid hormone but clomiphene citrate is a non-steroidal estrogen. The bioavailability of the drug is more as it is absorbed directly from the oral route. The Drug gets metabolized in the liver and excreted out by faeces.

Side effects

- During therapy poor quality of cervical mucus was found in one study.
- Pelvic discomfort, Nausea, Breastpain
- Ovarian Hyperstimulation
- Multiple Gestations. In one study twins occurred in 6.9% of pregnancies, triplets in 0.5% and quadruplets in 0.3% and quintuplets in0.13%
- Birth defect

Letrozole: Letrozole has more beneficial action than clomiphene citrate as it doesn't have negative impact on the endometrial lining and cervical mucous secretion. It is one of the recently identified ovulation inducing drugs. The drug is generally administered from 2-6thday. the dosage of drug is 2.5mg/day. It prevents multiple pregnancy, hyperstimulation and luteal phase defects are also not seen,

Human Gonadotrophins- among ovulation induction drugs, gonadotrophinsplay a major role. Among this human menopausal gonadotrophin is obtained from urine of menopausal women as their urine contains high levels of FSH and LH, as their follicular activity is depressed. These drugs directly act on the ovaries and causes stimulation of follicles, but has many untoward effects towards the health of the mother Ex. It may cause ovarian enlargement filled with cystic fluid and coagulation defects.

Regimens Combining Clomiphene and Gonadotrophins: In those women who are not responding to clomiphene citrate is given with combination of clomiphene citrate+ HMG.

Gonadotropin Releasing Hormone Therapy: Hypothalamus plays an important role in releasing FSH and LH from anterior pituitary. In women with hypothalamic dysfunction , gonadotrophin releasing hormone therapy is beneficial. These FHS and

LH is responsible for foliculogenesis, there are no major health hazards observed during administered of these drugs.

Bromocriptine Therapy for Ovulation Induction: High prolactin levels hamper proper GnrH pulsatile release. Bromocriptine is one of the dopamine agonists drugs. It suppresses the serum. Prolactin levels. The minor side effects are headache, Nasal congestion and Fatigue.

Progesterone for Therapy of Luteal Phase DefectS: even these are used for luteal phase defects.

Surgical methods to induce ovulation: Due to cystic lesions the ovaries size increase and Wedge resection helps in reducing the increased ovarian volume. About ½-1/3rd of the increased ovarian size can be reduced with Ovarian wedge resection surgery. Due to reduction of ovarian volume, there are chances of ovarian failure and post-operative adhesions to the adjacent structures.

Ovarian Laser Vaporization or Electro Cautery (Drilling ofTheovary):It is a drilling surgery where the several points of the ovarian tissue to get result similar to wedge resection without those complications.

DRUG REVIEW

पिप्पलि श्रुन्गवेरश्च मरिचं नागकेशरं ।

घ्रुतेन सह पानेन वन्ध्यापि लभते सुतम् ॥ 52 || Bhai ra. Yoni RogaChikitsa

For this experimental study the combination of Pippali, Sunti, Saricha & Nagakeshara is taken.

NAGAKESHARA[58]–

Introduction

Nagakeshara is a medium or large sized evergreen attractive tree and often its used part is stamen as several of its name Naagapushpa , Naagakeshara, Hemapushpaa and found at 500ft in eastern &western ghats.it is included under Elaadi, Vacaadi, Anjanaadi, & Priyangwaadi by Susruta and Vagbhatta. Looking forward to nighantus we can find many references regarding the drug Naagakeshara.

Naagakeshara i.e., (Mesua ferrea Linn) belongs to the family clusiaceae. The Sanskrit word Naagakeshara literally means that which is liked by elephant and Naaga.

Dourgandhyahara, Vishahara, Kushtagna, Shodhahara are the main Karmas of the drug Naagakeshara

Figure : 29 (a) Flower of nakeshara (b) Nagakeshara stamen

Taxonomical Classification

Common name – Messua ferrea Linn

Kingdom - Plantae

Subkingdom-Viridaeplantae

Phylum-Tracheophyta

Subphylum -Euphyllophytina

Class - Magnoliopsida

Subclass - Dillenidae

Order – Hypericales

Family – Clusiaceae

Subfamily-Kielmeyeroideae

Genus – Mesua

Species – Mesua ferrea

Division – Magnoliopsida

Nirukti

Etymology means the derivation of the word. This indicates the character of the drug and also gives some meaning about the drug related to the word.

One which is liked by snakes and elepahants is called as Nagakeshara

Synonyms

VERNACULAR NAMES

- Sanskrit – Naagakeshara, Naagapushpa, Champeya.
- English – Cobras saffron
- Hindi – Naagakashara, Gajapushpa
- Kannada – Naagasampige, Atta, Iruppumara, Kaanchana, Naagachampa
- Malayalam–Naagachampakam, Veila, Veluttachempakam, Nanga, Nangu, Periveinavu
- Telugu – Naagakesharamu, Gajapushpamu, Naagapushpu, Kaesharamu , Kinjalkamu, Thadinangu
- Tamil – Veluttachampakam, Cherunaagappu, Sirunaagappu, Irulkarunangu, Malainangu, Mannainangu, Naagachampakam
- Bengali - Naagesharkongini – Naagchampe

- Urdu – Narmishka

GANA VARGEEKARANA

Surutha Samhita	EladiVarga,VachadiVarga,AnjanadiVarga Priyanvadivarga
AstangaSangraha and AstangaHridaya	EladiVarga,VachadiVarga,AnjanadiVarga Priyanvadivarga
Dhnvantari Nighantu	ShatupushpadiVarga
Shodala Nighantu	ShatupushpadiVarga
Raja Nighantu	PipplyadiVarga
Bhavaprakashaya	Karpuradivarga
Kaidarya Nighantu	Aoushadivarga
Bhavaprakasha Nighantu	Chaturjata

Table no 4 - Ganavrageekarana

History

Nagakeshara is a drug used from ancient times, also we get the references of this drug in Bruhatthriie, Charakasamhita, Susrutasamhita & Vagbhattasamhita and also in almost all the nighantus.

The antecedancy of Nagakeshara dates back to the Vedic period. It is mentioned in Adharvavedaparisishta (AP 70/7/1).In the Bruhatthrai text, the term Nagakeshara is used for very few times while other synonyms are more used. Ahapushpa & Ahikeshara are the synonyms used by Susruta & Vagbhattarespectively. Charaka did not mentioned it under 50 vargas. However, he mentioned it as one of the ingredients of Kanakaarishta, as external application for visarpa and as an ingredient in baalataila. Susruta & Vagbhatta have used it respectively.

Among laghuthrais Sharangadhara & Baavamisra have delineated Nagakeshara several times. Almost all the nighantus have described the properties and indications of Nagakeshara. It is mentioned as Chaampeyah in Amarakosha and is known as Nagachampa.

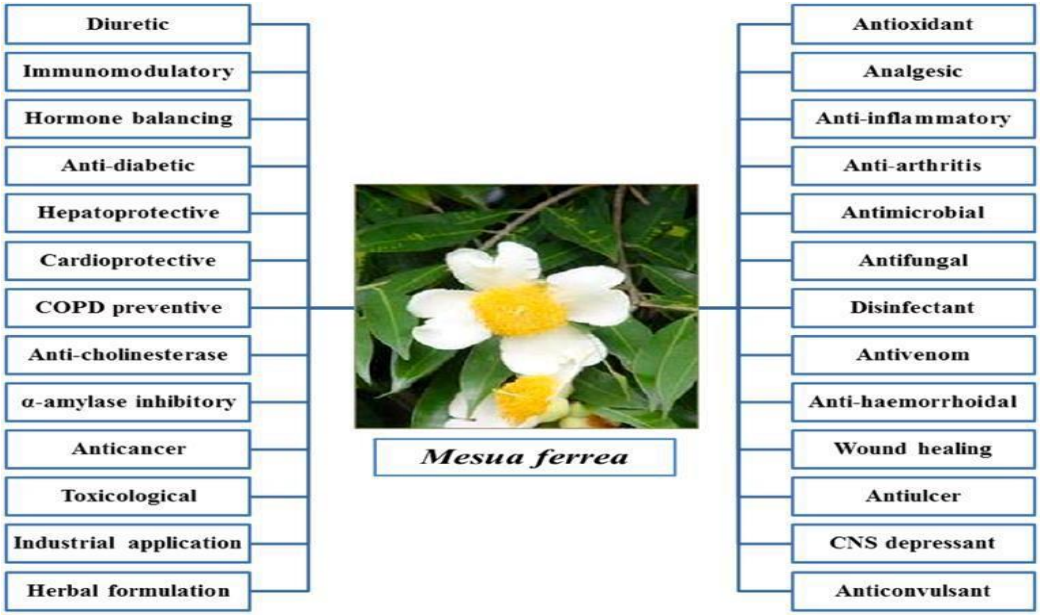


Figure 30. Pharmacological actions of Nagakeshara

Habitat In India

- Mountains of eastern Himalayas &Westernghats
- East &WestBengal
- Assam
- Evergreen forests of north Kanara &SouthKanara
- Andamans
- SouthGoa
- NorthKarnataka
- SouthIndia
- Chickamanglore,Coorg,Mysore,Shimoga

Global

- Nepal
- Bangladesh
- Srilanka
- Myanmar
- Malaysia
- Indonesia
- VeitnamCambodia
- Thailand

MORPHOLOGY OFNAGAKESHARA

- It is a medium sized or large evergreen tree with short trunk and dark red heartwood ;often buttressed at the base.
- Leaves → Simple, Opposite, Aute- Acuminate apex, Coriaceous, Lanceolate(5.0-15.0 × 3.5-4 c.m).Upper side is glabrous and lower side is covered with a white waxy powder ie,glaucous. Immature leaves are red incolour.
- Flowers → white or orange, large, fragrant,8-10 c.m in diameter. Solitary or in Clusters.
- Stamena → Stamens may sterile in female flowers (this may be the reason for it to call as punnaaga). Stamens are numerous,golden yellow colored,shorter than petals.
- Fruit → 2-5 c.m long, ovoid with persistantcalyx
- Seeds → 1-4 seeded, Shining & dark brown with oily and fleshy cotyledons.
- Flowering – February to April
  Fruiting- September to October

Figure 31(a) Flower of nakeshara (b) nagakeshara stamen

Microscopic Features

BUDS → Microscopic features include the presence of cortical fibres, numerous resin canals & calcium oxalate crystals in the cortex and pith of the pedical.

STAMENS & FLOWERS → The atamans are numerous with short filaments and some what thick elongated anther lobes. Stamens and flowers appears blackish brown in colour. Under UVlight the powdered stamen appears olive green while the flowers appear purplish brown, using nitrocellulose in amylacetate.

Powdered stamen can be distinguished by presence of copper colored filaments, striated cuticular layers of anthers, a characteristic endothelial layer, calcium oxalate crystals, spiral vessels &spherical to oblong pollengrains with granular exine having three germinal pores & furrows.

Stamens have long hairy filaments and somewhat thick , tetra sporangiate, elongated anthers, some fused stamens exhibit vascular strands also. Occasionally the fusion may involve the anther lobes as well. In such cases,however,fusion of the anther lobes is not complete.

On anther lobes long unicellular, multicellular, uniseriate and stellate trichomes are found, however on filament only unicellular, multicellular, unicerate trichomes are observed.The cells of connective are lignified and pappillate. TS passing through anther lobes shows tetrasporangiate condition, single layered, elongated papillate epidermis interrupted by unicellular,multicellular, unicerate trichome followed by single layered thick walled fibrous endothecium near the dehiscence line but become gradually multicellular towards the connective cells are parenchymatous and contain spiral bud of lignified thickening appearing as heads on the walls in surface view. A vascular strand contains annular to spiral vessel.In surface view the cells of the epidermis of the filaments show straight anticlinal walls and anicocyticsomata.

Rasa Panchaka

	RASA			**GUNA**		**VIRYA**	**VIPAKA**	**DOSHAGNA**
	TIKTA	KASHAYA	LAGHU	RUKSHA	TIKSHNA	USHNA	KATU	
B.P	-	+	+	+	-	+	+	V-K SHAMAKA
DHA.NI	+	-	+	-	-	+	+	KAPHAHARA
RA.NI	+	-	+	-	-	+	+	KAPHAHARA
MA.NI	+	-	+	+	-	+	+	KAPHAHARA
KAI.NI	-	+	+	+	+	+	+	KAPHAHARA

Table no 5- Rasa Panchaka of Nagakeshara according to different authors

ROGAGHNATA

Kandu	Kushta	Chardhi
Trushna	Hrullasa	Jwara
Visha	Dourgandhya	Visarpa
Shotha	Basthiruk	Swaasa
Kaasa	Brshas	

PHYTO CHEMISTRY

Nagakeshara contains

- Mesuol
- Essential oil & oleo resin fromflowers
- Mesuaferone A & B fromstamens
- Mesuaferrol
- Leucoanthocyanidin
- Mesuone
- Mammeigin
- Mesuagin
- Euxanthone
- FerruolA&B
- Guttiferol&ferraxanthone
- Cuxanthone
- Volatile oil
- Resins
- α –amyrin
- β –amyrin
- β – sitosterol Mesuanicacid

Adultration And Substitution

Adulteration and substitution of the herbal drugs is the burning problem in herbal history. The deforestation and extinction of many species and in correct identification of many plants has resulted in adulteration and substitution of raw drugs. Concept of substitution ages back and in Ayurveda we can find this in the treatise of Bhavaprakasha and Yadavji Trikamji Aachaarya. Adulteration – substitution of the original crude drugs partially or fully with other substances which is either free from or inferior in the therapeutic and chemical properties.

Substitution – Crude drugs are substituted with the inferior commercial varieties are used as adulterant which may or may not have any therapeutic potential as that of original drug.

There is no controversy in the originaltext books where its utility is limited. With the advent of Chathurjaathaka and increased utilization during nigantu period might

resulted in adulteration in the commercial markets. Nagakeshara is always described as one variety. Sivadatta gave more confusion by quoting that the keshara of punnaagavriksha as Nagakeshara.It is very clear that punnaga & Nagakeshara are two different trees. It appears thet the confusion is mainly because both Nagakeshara and Punnaga are having closely related name. Naagakeshara – Mesua ferrea Linn Padma keshara – OchrocarpuslongifoliusBenth& Hook (Ratan Nagakeshara). Third variety ie, Kaala or Karu Nagakeshara. It is black variety. This variety is found to be common substitute or adulterant for Nagakeshara.Kala nagakeshara – Unripe fruits of Cinnamomunwightii Nagakeshara sold in the South Indian bazaars is of two types i.e., Malabar nagakeshara – Unripe fruits of Dilleniapentagyna Nattukeshara – Unripe fruits of Cinnamumwightii.

Posology

A/C TO Rajanighantupushpa or parag 2 to 10 gms A/C TO Dr Bapalal Twakkwatha 50 to 100 ml Beejachurna 3 grams**,** Taila 2 to 5 drops**,** A/C to P V Shramapumkesarachurna 1 to 2 grams.

Research Article

Original Research paper: An Ayurvedic Review of Nagakesara (Mesua ferra) from Samhita amd Nighantu.Samhita and Nighantu are the basic literature for understanding and identification of different medicinal plants. On review of Nagakesara in different samhita and nighantu it was found that it has Garbhasthapaka Karma.

Study of aqueous extract of M.Ferrea flower &C. Papaya seed administered orally to female albino rats increased and decreased the hormones, suggesting that these two plants extracts might boost up the general health of the animal. As the rat is a mammal with the estrus cycle is same with that of humans, hence this plant can be used in human beings.

TRIKATU

- **PIPPALI**[59]

Hindu mythology reveals that Pippali has its origin during Samudramanthana along with Amrita one context from Jaiminiya Brahamana delineate that the some of saint vasita consumed Pippali to attain health and wealth. In Atharva Veda Pippali is mentioned as Rasayanaoushadhi.

P. longum is an indigenously growing plant in India and is also cultivated in the tropical and subtropical regions of Asia and Pacific islands. It is usually cultivated for its fruit which is dried and used as a spice. The plant grows into a shrub with large woody roots, numerous creeping and jointed stems that are thickened at the nodes. Leaves are without stipules and spreading in nature. Fruits are small, oval shaped berries and grow as spikes that are collected after maturation. Dried form of these spikes makes Pippali while the root radix is known as Pippalimula. The dietary piperine is known for its bioavailability and digestion enhancing properties. *In vitro* studies have shown the role of piperine in relieving oxidative stress by uenching free radicals and reactive oxygen species. While it is known to act as an anti-mutagenic and anti-tumor agent , anti- diarrheic and anti-dysenteric properties of this spice enhance its medicinal value. The pharmacological properties of this plant also include antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, anti-microbial, anti-platelet, anti-hyperlipidemic, analgesic, anti-depressant, anti-amoebic, anti-obesity, radioprotective, cardioprotective and anti- fungal. Methanolic extract of this fruit has been reported to be involved in memory repair and improving memory performance by an *in vitro* model. Clinical studies have revealed the efficacy of this plant in the treatment of bronchial asthma in children. Anti-diabetic activity of the roots has also been reported. It is widely used as an important constituent in various Ayurvedic medicines to cure diseases like leprosy and tuberculosis and is also used in the treatment of cough, dyspnea, cardiac and spleen disorders, chronic-fever, gout, rheumatic painetc.

Acts as a rasayana, ghee prepared with pippali and milk will be useful.

Vernacular name-

- Bengali-Piplamor
- English- Indian long pepper
- Hindi- Pipli
- Kannada - Hippali
- Malayalam - Thippali
- Sanskrit - Pippali
- Tamil - Thippli
- Telegu - Pippallu
- Urdu - Pippal

Toxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnolipsida

Order : Piperales

Family : Piperaceae genus : Piper species : P. Nigrum

Botanical name : Piper longum

GANA VARGEEKARANA-

Caraka	Deepaniya , Kanthya, Asthanopaga, Sitaprashamana,Sulaprashamana, Kasahara , Hikkanigrahana ,Trptighna
Susrutha	Pippalyadi , Trayushana , Amalakyadi , Shirovirechana
Vagbhata	Pippalyadi

Table no 6: Ganavargikrana of pippali according to different authors

Habitat

This is grown in Bengal, Bihar, Assam, Nepal, Bhutan, Singapore.

Found throughout the hotter parts of india, west coast and western ghats.

Chiefly in Haridwar, Lachiwal, Doiwala, Dehradun, in Tarai-Bhabar of Almoradistict, haldwani and ghonti in tehri forests.

Grown more in uttar Pradesh like Bengal, Bihar, Assam, Nepal, Malaysia, Indonesia, Singapore, srilanka, nicobar island etc....

Figure 32- Dry pippali

MORPHOLOGY

Pippali is an aromatic perennial twinner grows upto an height of 1mt height.

STEM: numerous, prostate(not climbing), much branched, stout, cylindrical, thickened above nodes finely pubescent.

LEAVES: leaves are 2-3 inch long. The older leaves are dentate, dark in color and hart shaped. The younger leaf is ovate in shape and contains 5mins on them.

FLOWER: are monoecous and male and female flowers are borne on different plants. Male flower stalk is about 1-inch long and female flower stalk is ½ - 1 inch long. It flowers in rainy season.

Male flower: stalk is about 1-3inch long. Female flower: stalk is ½ - 1 inch long.

Fruit : is long when it ripes it attains red color and when it dries it attains black color. It is one inch in diameter. Fruits are seen in early winter.

Types- black pepper, white pepper, green pepper,

Orange and red pepper.

GUNA-KARMA

	KAIDEVA NIGHANTU	BHAV PRAKASH
Guna	Guru, Madhura, Snigdha	Laghu, Snigdha, Tikshna
Rasa	Katu	Katu
Virya	Ushna	Anushna
Vipaka	Katu	Madhura

Table no 7:Gunadi krama of pippali according to different authors

Posology

Choorna – 0.5-1 gm Swarasa – 10-20 ml Kwatha – 50-100 ml RESEARCH

Articles

Critical review on various ethonomedical and pharmacological aspects of Piper longum (long pepper or pippali) Department of botany and microbiology2018

Piper longum possesses several pharmacological properties like antibacterial, antifungal, insecticidal, antinuclear, antiplalet, antiamoebic, hepatoprotective, adulticidal,anti-obesity,antidepressant,anticancer,antiasthamaticetc. P.longumpossesss starch, protein and Alkaloids, volatile oils, saponins, carbohydrates and amygdalin.

MARICHA[60]

Introduction To Maricha

Botanically identified as Piper nigrum belongs to piperaecae. Maricha is a stoot climber trailing and rooting of fine nodes upto 10m.

It is distributed more in Malabar region, Travancore, Allappey, Ernakulam, Konken, Kanyakumari, Southern India, Kerala. It is one of the main ingredients of Trikatu.

Vellaja, Krishna, Dharmapettina are some of the classical synonyms given for maricha. Fruit is the part used of this plant.

Nirukti

It acts against the poison or poisonous effects.

Vernacular Names

- English:- Black pepper, Common pepper, Pepper, Filfiegrid.
- Hindi :- Kaalimirch, Mirch, Gulmirch,
- Kannada:- Kaglumenasa, Ollimonsu, Mirimanasu, Karemanasu, Moluvukodi, Mirilu.
- Malayalam:- Kurumulaku, Lada,Mallamulaku.
- Marathi:- Mira,Kaalimiri.
- Punjab :- Golmirch,Habush.
- Tamil:-Pippil,
- Telugu :- Miniyral,Maarichem.

Taxonomicalidentification

- Kingdom –plantae
- Division –magnolipophyta
- Class -magnolipophyta
- Order –pepper
- Family–piperaceae
- Genus–piper

Morphology

It is a much branched trailing or climbing shrub. Reenes a height of 20-30 feet.The old stems woody, rough, the younger much branched dichotomously, smooth, thickened, at the joints, from which arise adventitious rootlets when attached themselves to neighboring supports or root in the soil.

Leaves :Leaves are simple alternate, cordate, broadly ovate. Which is without stipules widely spreading on rounded petioles about half inch long, blade 4-6 inches long, acute at base and apex. 5-9 nerved dark green below and shining above.

Flowers: Flower are minute in spikes of variable length, usually dioecious, but the female often bears anther and the male a pisillode.

Fruit: Ovid or globulose one seeded, bright red when ripen.

Seeds: Globulose, testa thin, perisperm hard and white.

Rasa Panchaka

Rasa	Katu, Tikta
Guna	Laghu, Teekshna
Vipaka	Katu
Veerya	Ushna
Doshakarma	KaphavataShamaka

Table no 8:Rasa Panchakaof Maricha.

Samsthinika karma

External action – Rakthothkshakava, Lekhana and Chedana

Internal action – Nadivahasamthina, Nadiuttejaka and Bilya.

Pachanasamsthana

Dipana, Pachana, Anulomana, Agnidipaka, Rucikara, Yakruthuthejaka, Krimighnatha.

Rakthavahasamsthana

Uttejaka

Swasasamsthana

Kaphagna, Kaphanissavaka

Mutravahasamsthana

Mutrindriyauttejaka, Mootraka

Prajananasamsthana

Uttejaka and Arthavajeneka

Srothodhaka

PHYTO CHEMISTRY

Maricha contains…

- Piperine:9-10%
- Piperidine:5%
- Organic matters:7%
- Protein:11.5%
- Fat:7%
- Carbohydrate:41.2%
- Calcium: 460
- Iro:16.8%
- Thiamine:0.06%

- Riboflavin: 0.14%
- Nicotinic acid:1.4%
- Essential volatile oil:1.2%
- Organic matter:7%
- Minerals:4.4%
- Vitamins
- Piperitine
- cerinine
- Lianin
- Starch

Researches

- Anti-bacterial constituents form the basis of pipernigrum.
- According to research administration of methanolic extract of piper nigrum leaf
- An alkololia isolated from the stems of piper laetsem piper lactispermamide,
- laetispiene is acting as on antidepressant
- The fruit extract reported to be inhibitory of e-coli acrobateraerotes, leeser, Stephenetc(1957).
- Piper nigra exhibits anti-bacterial and anti tumour activities against pseudo, aroginosaandalciligens(1987)
- Its insecterdal activity isreported(1984).

Adulterant

Black pepper is not liable to adulteration in its entire state, but when powdered it is frequently mixed with various kinds of starch, mustard husks, in seed, capsicum and papaya seed.

SHUNTI (ARDRAKA)[61]

The botanical name for Ginger – Zingiber Officinale – was given by the Swedish botanist Innaeus . It comes from the sanskrit word of sringavera , which means *'shaped like a horn'.* Theterm officinale simply means that the plant is commonly available & is useful to humans in medicine as a food. An ancient remedy, ginger is used for many purposes, most notably perhaps for nausea as a stimulant it helps to improve peripheral circulation. According to Charaka and Sushruta it is a good vrishyadravya it is also a ruchikara and deepana. Later Nigantus also mentions its role in management of pandu, vatodar, kaphanila hara, shleepada Bhava Prakash also mentioned it as a good Pachakadravya.

आद्राकम्आद्रायनतनजह्वभम्(नि. आ.)

It will provide moisture to tongue i.e., useful in treatment of dryness of mouth.
It promotes growth of human body.
It almost cures every disease.
It can be cultivated in all over the world.
It posseses several sringas (germinating buds) on its surface.

Vernacular Names

- English- Ginger
- Hindi - Adaraka
- Bengali- Adrak
- Gujarathi- Suntha
- Kannada- Ardraka
- Marathi- Ardrak
- Punjabi- Adrak
- Tamil - Sukku
- Telegu - Ardrakamu
- Assam - Adasuth
- Oriya - Sunthi
- Urudu - Adrak
- Malayalam- Inji

Synonms

- Ardraka
- Shringavera
- Vishva
- Mahaaoushadha
- Nagara
- Katukanda
- Aardaknagaram
- Shringaveshardrakam
- Vishvabheshajya
- Katutkata
- Katubhadra
- Aardraka

Gana Vargeekarana

CHARAKA	Arshoghna, Deepaniya, TrptighnaSulaprashamana, Trshnanigrahana
SUSRUTHA	Pippalyadi , trikatu
VAGBHATA	Pippalyadi

Table no 9: Ganavargeekarana of aadraka according to different authors

Taxonomical Identification

Common name - Ginger

Kingdom - Plantae

Superdivision- Spermatophyta

Subkingdom - Tracheobionta

Class - Liliopsida

Subclass - Gingiberidae

Order - Zingiberates

Family - Zingiberaceae

Genus - Zingiber

Species - Officinale

Habitat : Throughout tropical Asia &India also in region of sub Himalayan tracts and own wild in some places in Western Ghats . On the large scale in warm & moist regions. Also cultivated in West Indies , Jamaica , Africa.

Morphology

It's an erect perennial herb with aromatic rhizome. Stem –erect leafy 15-150 cm tall

Leaves-sessile, linear –lanceolate, 10-25x*105-30cm.

Narrowed to the base, acute or the acuminate, sheath 10-15cm long.

Flowers – greenish milk a small dark purple lip ,in oblong cylindrical spikes ensheathed in few scarious ,glaborous bracts ,4 to 7 cm long.

Stamens- dark white coloured

Fruits – oblong capsules

Rhizome – white to yellowish brown in colour ,irregularly branched somewhat annulated laterally flattened

Flowering & fruting during july – september

Varieties

According to habitat & processing ,there are many varieties

- Dry ginger is smoky incolour
- White coloured ginger is found in south India which is used on practice peeled rhizome boiledin milk and dried is called as Dudhiyasuntha

In Kaiyadevanighantu, it is described that ardranagaram & ardrakam separately the former is fresh ginger, while the later is dry ginger. In Amarkosa dry ginger is denoted as Nagara & Visvabhesajyaetc, while describing fresh ginger as Ardraka & Sringaberaetc

Figure 33 (a) Plant of shunti (b) tubers of shunti

Guna Karma

RASA	Katu
GUNA	Guru , ruksha , Tikshna
VIRYA	Usna
VIPAKA	Madhura
KARMA	Sula hara , Admana hara , Atisaraghna, kasaswasa hara, Hrdrogaghna , Vibanda hara , Jwaraghna

Table no 10- Guna karma of Shunti

Karma

The various karmas are described as follows:

- Samsthanika Karma Bahya- acts as Sheethaprashmana, Shothhara & Vedanasthapana.
    - NaadiSamasthanika – acts as energizer & Vat Shamaka
    - Pachanasamasthana – Deepana Pachana & Vatanulomana
    - Raktavahasamsthana –Sothahara & Raktashodana
    - Swasansamsthana- Kaphagna & Swasahara
    - Prajananasamsthana- Aphrodisiac
    - Taapkarma- Jwarghnata, Sheethaprashmana
    - Satmeekaran- it acts as the best Aampachanadravya

Rogagnata

- Jwaragna
- Shotha
- Amavata
- Gulma
- Shleepada
- Vrishanavata
- Shiraroga
- Vatavyadhi
- Arshas
- kshatksheena
- Karnashula
- Ajirna
- Agnimandya
- Sheetapitta
- Vrischikavisha
- Atisara
- Udararoga
- Kamala
- Hrudyaroga
- Aruchi
- Kaasa
- Murcha

PART USED: It depends upon the major chemical constituents on that very

particular part.In the drug Ardraka ,the part used is Scraped & dried rhizomes as well as the green ones

Phytochemistry

- Total ash- Not more than 8%, Acid insoluble ash- Not more than1%
- Alcohol soluble extractive – Not less than5%
- Water soluble extractive- Not less than 2%
- Moisture content – Not more than90%
- Chemical constituents
- Heptane
- Octane
- Isovaleraldehyde
- Nonanal
- Ethylepineme
- Camphene
- B-pinene
- A-curcumene
- B- D-Curcumene
- Geraniol
- Gingerol
- Zingiberenes
- Zingiberol
- Zingerone
- Ginger glycdipids A,B &C
- Gingerone B &C
- Dosage of Ardraka
- Decoction : 15 ml
- Powder: 0.3-1g
- Oleoresin : 15- 60mg

Posilogy : Churna:1-2gm , Ardrakaswarasa : 5-10ml

THERAPEUTIC USES

- Antispasmodic : It relaxes all types of muscle
- Aromatic: Ginger's aroma, flavour and warmt help to stimulate thedigestivesystem
- Carminative: The volatile oils in ginger relax the stomach and stimulate peristalsis thereby supporting digestion and reducinggas.
- Diaphoretic : It induces perspiration and the elimination of the toxins through the skin
- Rubefacient- Applied to the skin, ginger stimulates and dilates the blood capillaries, increasing circulation.
- Sialogogue: It promotes the secretion of saliva
- Stimulant: As, a circulatory aid, it supports and speeds up the body's physiological systems.
- Jaladosa- Ardraka and Yavaksara are taken with lukeworm water.
- Pratisyaya- Ardraka is given that with milk
- Kaphaja Arsa- Ardraka and Kulutha are used
- Murcha- Ardrakaswarass is used as Nasya.

There are several commercial varieties of ginger derived from Z. Officinale.

Apart from these some types are derived from other species i.e., Japanese ginger is obtained from Z. Mioga. Rosc. and Martinique ginger from Z. ZerombetRosc. The rhizomes of Z.casummarRoxb. are sometimes used as substitute to Z. Officinale.

Research Article

- Gingiber might improve female infertility: A rat model
- Conclusion: increase in the antral follicle count and ovarian stromal 100mg/5 day treatment subgroup indicate that gingiber have positive effects on folliculogenesis. In short term with low dose in additional ginger may enhance implantation in rats in long term low dose.
- An international Journal of Pharmaceutical sciences Female infertility: A major problem worldwide and its management in Ayurveda. All major active ingredients of Z. officinale suchas Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols have antioxidant activity. Besides that, ginger has a useful effect on folliculogenesis and ovumquality.
- Ginger has been the focus several scientific studies, showing that it works on several levels in the treatment of migraine. It is powerfully anti-inflammatory, anti-nociceptive (pain reliever), anti- emetic (nausea) and it suppresses the release of many chemicals involved in the body's immune system response to migrainetriggers.
- Recent studies also show that it suppresses the release of CGRP a key peptide in the pain of a migraine. Another potential therapeutic effect of ginger on migraine can be explained in part by the role ginger plays in enhancing estrogen levels. Several factors explain the differences in the prevalence of migraine between women and men during a woman's reproductive years. For instance, studies show differences in headache characteristics and the anatomy of the central nervous system. A significant amount of research suggests that hormones are one of the leading factors that make women more susceptible to migraines. More specifically, studies showthat migraines are significantly influenced by estrogen concentrations, with lower estrogen levels leading to a higher risk for migraines

MODERN DRUG REVIEW

PROGESTINS [62]

Progestins are the synthetic form of hormoneprogesterone. These group of drugs were first introduced in 1934, and Ethisterone is the first progestin used medically in the year 1939. Many other progestins like Norethisterone was developed as birth control pills . These progestins helps in achieving pregnancy by converting the estrogen primed endometrium to secretory endometrium.

Progestins have two sources –

- Natural – they are derived from cholesterol ,Progesterone 21 which is a carbon steroid. they are secreted from the corpus luteum by the action of
- Synthetic progestins- They are derived either from the progesteroneornortestosterone.

Initially the orally active derivative of testosterone called ethosterone was prepared due to discovery of oral potency of ethyl group substitution. In 1951, Noresthidrone was discovered by removing the 19-carbon from the ethisterone. These were progesterone agents.

The progesterone derivatives of testosterone are called 19-norestosterones.

They are the modification or analogs of norethindrone, levonorgestrel . They have an ethinly group instead of extending methyl group from c-13. They have receptors for progesterone, androgens and glucocorticoids.

Mode of Action

Progesterone receptors are present in the nucleus of the target organs in female genital tract, breast, pituitary glands, CNS glands. They undergo dimerization and attaches to the PRE- progesterone receptor element and they regulate transcription. These progestens undergo first pass metabolism in liver which depends on the binding capacity to the SHBG sex hormone binding globulin. Among the progestens, the norgestimate have weak SHBG binding capacity and hence they have greatest 1st pass metabolism. Ethyl estradiol has Entero-hepatic circulation .

Pharmacokinetics

Administration- Progestins drugs are administered in various ways. They have high first pass metabolism in Liver. Synthetic progestins are orally active and is metabolized slowly have plasma t½ ranging from 8hrs-24hrs. The plasma concentrations of progestens rise over time. The concentration of free drug which is unbound which diffuses into bloodstream into the targets organs to bind in receptors present in the hypothalamus, pituitary, mammary gland and uterus. The activity is best measured as the affinity for the progesterone receptor which suppress the luteinizing hormone (LH) secretion or which stimulates the endometrial development. The changes are due to the stimulation of estrogen of SHBG (Serum hormone binding globulin) production, binding of progestogen SHBG and decreased SHBG concentrations and they in turndecreasethe progesterone concentrations over time

Distribution – Norethisterone has plasma binding around 97% which is bound to albumin 61 % and to SHBG 36%.

Metabolism [63] **-**metabolism of norethisterone is similar to testosterone metabolism. Norethisterone has an elimination of ½ life of 5.2 to 12.8hours.

Uses of Progestins-

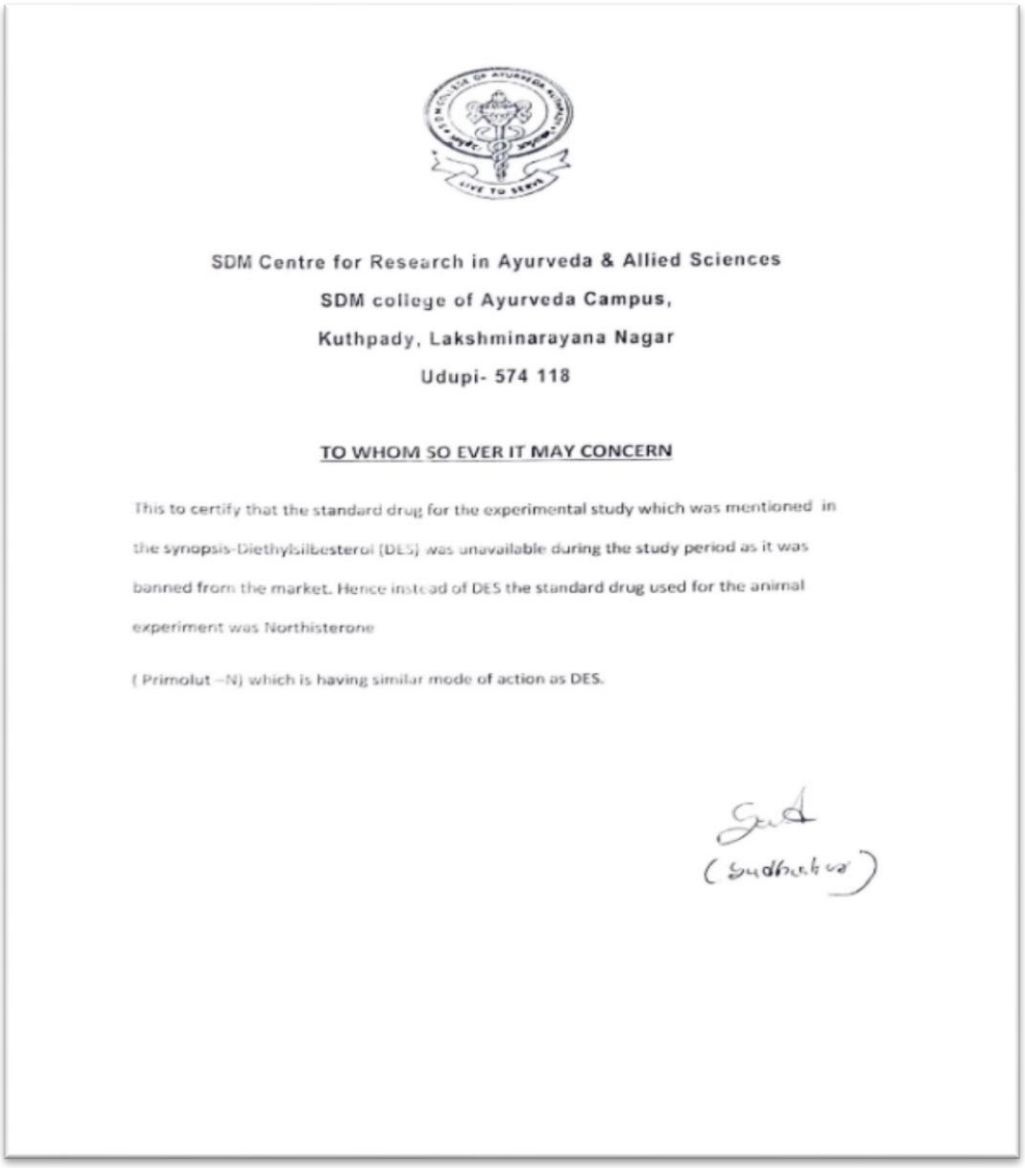
- It is used for hormone replacement treatment in women with premature ovarian failure, menopausal women and secondary or tertiary hypogonadism. They are given along with estrogens to post-menopausal women.
- Progestins are used as a continuous or cyclic regimen when the women are havingmenstrual bleeding.
- Medroxyprogesterone acetate is given orally for 25days. initially 5-6days no hormones given and withdrawal bleeding is expected to occur. Continuous administration doesn't trigger withdrawal bleeding, but only intermittent spottingis seen.

CHAPTER 6

RESEARCH METHODOLOGY

As Diethylstilbestrol is banned from the market, Norethisterone was taken as substitute.

certificate of change of drug to Norethisterone



SDM Centre for Research in Ayurveda & Allied Sciences

SDM college of Ayurveda Campus,

Kuthpady, Lakshminarayana Nagar

Udupi- 574 118

TO WHOM SO EVER IT MAY CONCERN

This to certify that the standard drug for the experimental study which was mentioned in the synopsis-Diethylsilbesterol (DES) was unavailable during the study period as it was banned from the market. Hence instead of DES the standard drug used for the animal experiment was Norethisterone

(Primolut –N) which is having similar mode of action as DES.

In this chapter methodology of all the 3 objectives are discussed.

METHODOLOGY OF OBJECTIVE -1

Standardization and HPTLC of Trikatu Churna

PHYTOCHEMICAL ANALYSIS

Acid soluble

In a tared evaporating dish 10g of sample was placed and dried at 105 C . The sample was kept in hot air oven for 5 hrs. and weighed . The drying was stopped when the differencing in the weight was not more than 0.01 by cooling in desiccators then percentage of moisture in the sample was calculated in regards to weight of sample.

Total Ash

In a tared platinum crucible 2grams of sample was incinerated at 450 C . The obtained carbon free ash is collected and percentage of ash was calculated.

Acid insoluble ash

To the ash in the crucible 25ml of diluted hydrochloric acid was added and boiled. The insoluble matter was collected on ash free filter paper and washed by hot water till the filtrate became neutral. Then the filter paper with insoluble matter was transferred to the original crucible , dried on a hot plate and ignited to constant weight. Then the residue was allowed to cool in suitable desiccator for 30mins and weighed immediately. The content of acid insoluble ash was calculated with reference to air dried drug.

Alcohol soluble extraction –

4g of the sample was weighed and kept in a glass stoppered flask. To this, 100ml of distilled alcohol (95%) was added. It was shaken occasionally for about 6hrs and allowed to stand for 18hrs.Later rapid filtration was done to ensure that solvent is not lost during filtration and 25ml of the filtrate was pipetted out in a 100ml pre-weighed beaker .This

was kept in water bath for evaporation to dryness and in hot air oven for 6hrs at 105°C. It was cooled in desiccator for 30mins and weighed again . the percentage of alcohol extractable matter of the sample was calculated. The experiment was repeated again and the average value of the findings was considered.

Water soluble extraction-

4g of the sample was weighed and kept in a glass stoppered flask. To this, 100ml of distilled water was added and shaken occasionally for about 6 hrs. it was allowed to stand for 18hrs and rapid filtration was done and care was taken to ensure that their solvent was no lost during filteration.25ml of the filtrate was pipetted out in a 100ml of pre-weighed beaker. It was allowed to evaporate to dryness in water bath and was kept in hot air oven for 6hrs at 105°C. it was later cooled in a desiccator and weighed again. The experiment was repeated again and the average value was taken.

PRELIMINARY PYTOCHEMICAL TESTS-

a) TESTS FOR ALKOLOIDS

Name of the test	Method of performing the test	Inference
1) Dragendroff's test	Extracts of the sample was taken and dissolved in alcohol . Drops of acetic acid & dragendroffs reagent was added and shaken well .	An orange red precipitate indicated the presence of alkaloids
2) Wagner's test	Extracts of the sample was taken and dissolved in acetic acid . Drops of Wagener's reagent was added.	A reddish- brown precipitate indicates the presence of alkaloids.
3) Mayer's test	Extracts pf the sample was taken and drops of Mayer's reagent was added.	A dull white precipitate indicates presence of alkaloids
4) Hager's test	Extracts of the sample was taken and dissolved in acetic acid. 3ml of Hager's reagent was added.	A yellow precipitate indicates presence of alkaloids.

Table no 11- Tests for Alkaloids

b) TEST FOR CARBOHYDRATES

Name of the test	Method of performing the test	Inference
1) Molisch's test	Extracts of the sample was taken and 1ml of α-naphthol solution and conc. sulphuric acid was added.	A violet colour was formed at the junction of two liquids.
2) Fehling's test	Extracts of the sample was taken and dissolved in acetic acid . Drops of Wagener's reagent was added.	A reddish- brown precipitate indicates the presence of alkaloids.
3) Benedict's test	Extracts pf the sample was taken and drops of Mayer's reagent was added.	A dull white precipitate indicates presence of alkaloids

Table no 12- Tests for Carbohydrates

(c.) TEST FOR STEROIDS

Name of the test	Method of performing the test	Inference
1) Libermann-Burchard's test	Extracts of the sample was dissolved in chloroform and 1ml of acetic acid and 1ml of acetic anhydride was added and heated in water bath and later cooled. Few drops of conc. Sulphuric acid were added	A bluish green fluorescence in the acid layer indicates steroids.
2) Salkowski's test	Extracts of the sample was dissolved in chloroform and equal quantity of sulphuric acid was added.	Bluish red colour in chloroform layer and green fluorescence in acid layer indicates presence of steroids.

Table no 13- Tests for Steroids

To the sample of extract, distilled water was added and shaken well.

Inference- stable froth formation indicates the presence of saponins

(e.) TEST FOR TANNINS

To the sample of extract, few drops of dilute solution of ferric chloride was added

Inference- dark blue colour shows presence of tannins

(f.) TEST FOR FLAVONOIDS

Name of the test	Method of performing the test	Inference
1) Shinoda's test	To the extract in alcohol, magnesium tumings and few drops of conc. Sulphuric acid were added and heated in the water bath.	Formation of red- pink colour indicates presence of flavonoids

Table no 14- Tests for Flavonoids

(f.) TEST FOR PHENOL

To the sample of extract in alcohol, 2 drops of alcoholic form of ferric chloride was added

Inference- bluish- black indicates the presence of phenols

(g.) TEST FOR COUMARINS

To the sample of extract in alcohol, 2 drops 2N sodium hydroxide solution was added

Inference- Dark yellow colour indicates the presence of coumarins

(h.) TEST FOR TRITERPENOIDS

The extract was warmed in tiny bits and drops of thionyl chloride.

Inference- pink colour indicates the presence of triterpenoids

(i.) TEST FOR CARBOXYLIC ACID

The extract was dissolved in water and treated with sodium bi-carbonate.

Inference- Brisk effervescence indicates the presence of carboxylic acid

(j.) TEST FOR AMINO ACID

The extract was added with ninhydrin reagent

Inference- a purple color indicates the presence of amino acid

(j.) TEST FOR RESINS

The extract of the sample was mixed with water and acetone.

Inference- turbidity indicates the presence of resins

(j.) TEST FOR QUINONS

The extract of the sample was mixed with 0.5% of sodium hydroxide

Inference- Deep coloration like pink purple or red indicates the presence of quinone

HPTLC

1gm of sample of Pippalyadi yoga sample was dissolved in 10.0ml of alcohol kept overnight and filtered. 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed under Toluene: Diethyl ether: Ethyl acetate (12.5: 4.3: 3.2). The developed plates were visualized in short UV, long UV and then derivatized with Vanillin sulphuric acid reagent subsequently scanned under UV 254nm, 366nm and

620nm (after derivatization). R_f, colour of the spots, densitometric scan and 3-D chromatograms were recorded.

Standardization and HPTLC of NAGAKESHARA CURNA-

Loss on drying at 105 degree C

In a tared evaporating dish 10g of sample was placed and dried at 105 C . The sample was kept in hot air oven for 5 hrs. and weighed . The drying was stopped when the differencing in the weight was not more than 0.01 by cooling in desiccators then percentage of moisture in the sample was calculated in regards to weight of sample.

Total Ash

In a tared platinum crucible 2grams of sample was incinerated at 450 C . The obtained carbon free ash is collected and percentage of ash was calculated.

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PRELIMINARY PYTOCHEMICAL TESTS-

c) TESTS FOR ALKOLOIDS

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6) Wagner's test	Extracts of the sample was taken and dissolved in acetic acid . Drops of Wagener's reagent was added.	A reddish- brown precipitate indicates the presence of alkaloids.
7) Mayer's test	Extracts pf the sample was taken and drops of Mayer's reagent was added.	A dull white precipitate indicates presence of alkaloids
8) Hager's test	Extracts of the sample was taken and dissolved in acetic acid. 3ml of Hager's reagent was added.	A yellow precipitate indicates presence of alkaloids.

Table no 15- Table for Alkaloids inference

d) TEST FOR CARBOHYDRATES

Name of the test	Method of performing the test	Inference
4) Molisch's test	Extracts of the sample was taken and 1ml of α-naphthol solution and conc. sulphuric acid was added.	A violet colour was formed at the junction of two liquids.
5) Fehling's test	Extracts of the sample was taken and dissolved in acetic acid . Drops of Wagener's reagent was added.	A reddish- brown precipitate indicates the presence of alkaloids.
6) Benedict's test	Extracts pf the sample was taken and drops of Mayer's reagent was added.	A dull white precipitate indicates presence of alkaloids

Table no 16- Table for Carbohydrates

(c.) TEST FOR STEROIDS

Name of the test	Method of performing the test	Inference
3) Libermann-Burchard's test	Extracts of the sample was dissolved in chloroform and 1ml of acetic acid and 1ml of acetic anhydride was added and heated in water bath and later cooled. Few drops of conc. Sulphuric acid were added	A bluish green fluorescence in the acid layer indicates steroids.
4) Salkowski's test	Extracts of the sample was dissolved in chloroform and equal quantity of sulphuric acid was added.	Bluish red colour in chloroform layer and green fluorescence in acid layer indicates presence of steroids.

Table no 17- Table for Steroids inference

(d.) TEST FOR SAPONINS

To the sample of extract, distilled water was added and shaken well.

Inference- stable froth formation indicates the presence of saponins

(e.) TEST FOR TANNINS

To the sample of extract, few drops of dilute solution of ferric chloride was added

Inference- dark blue colour shows presence of tannins

(f.) TEST FOR FLAVINOIDS

Name of the test	Method of performing the test	Inference
2) Shinoda's test	To the extract in alcohol, magnesium tumings and few drops of conc. Sulphuric acid were added and heated in the water bath.	Formation of red- pink colour indicates presence of flavonoids

Table no 18- Table for Flavonoids inference

(f.) TEST FOR PHENOL

To the sample of extract in alcohol, 2 drops of alcoholic form of ferric chloride was added

Inference- bluish- black indicates the presence of phenols

(g.) TEST FOR COUMARINS

To the sample of extract in alcohol, 2 drops 2N sodium hydroxide solution was added

Inference- Dark yellow colour indicates the presence of coumarins

(h.) TEST FOR TRITERPENOIDS

The extract was warmed in tin bits and drops of thionyl chloride.

Inference- pink colour indicates the presence of tritepenoids

(i.) TEST FOR CARBOXYLIC ACID

The extract was dissolved in water and treated with sodium bi-carbonate.

Inference- Brisk effervescence indicates the presence of carboxylic acid

(j.) TEST FOR AMINO ACID

The extract was added with ninhydrine reagent

Inference- a purple color indicates the presence of amino acid

(j.) TEST FOR RESINS

The extract of the sample was mixed with water and acetone.

Inference- turbidity indicates the presence of resins

(j.) TEST FOR QUINONS

The extract of the sample was mixed with 0.5% of sodium hydroxide

Inference- Deep colouration like pink purple or red indicates the presence of quinone

HPTLC

1gm of sample of Nagakeshara Churna sample was dissolved in 10.0ml of alcohol kept overnight and filtered. 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed under Toluene: Diethyl ether: Ethyl acetate (12.5: 4.3: 3.2). The

developed plates were visualized in short UV, long UV and then derivatized with Vanillin sulphuric acid reagent subsequently scanned under UV 254nm, 366nm and 620nm (after derivatization). R_f, color of the spots, densitometric scan and 3-D chromatograms were recorded.

METHODOLOGY OF OBJECTIVE 2

ACUTE TOXICITY STUDY

TEST CONDITIONS

1. Animal species : Rats

2. Strain **:** Wistar albino

3. Source **:** Animal house attached to SDM Research Centre, SDM Ayurveda College Udyavara

4. Selection **:** A total of 5 healthy either sex of body weight 150-200g

Rats were selected according to AOT software .

5. Acclimatization period **:** All the selected animals were kept under acclimatization for 7 days before dosing.

6. Numbering and identification **:** The animal was marked with saturated Picric acid solution in water for proper identification. The marking within the cages are as follows.

Animal number	Marking
1	Head
2	Neck
3	Middle of the back
4	Base of the tail
5	No mark

Table no 19- Number and identification of rats

The group number, animal number and sex of the animal were identified with the help of cage cards, as presented in the following table.

Sl. no	Identification of animals	Desired dose (according to AOT)	Body weight (grams)	Calculated dose (ml)
1	Head	175mg/kg	158	1.58
2	Neck	550mg/kg	162	1.62
3	Back	2000mg/kg	156	1.56
4	Base of the tail	2000mg/kg	160	1.6
5	No mark	2000mg/kg	154	1.54

Table no 20- Acute toxicity study

Husbandry condition:

1. **Housing :** Rats were housed in each cage of poly propylene with stainless steel top grill. The dry paddy husk was used as bedding material and was changed every morning
2. **Environment :** The animal was exposed to 12 hours light and 12 hours dark cycle with the relative humidity 50 to 70 % and the ambient temperature was 22 ± 03®c.
3. **Diet :** Amruth brand rat pellet feed supplied by Pranav Agro Ltd, was provided throughout the study period except on previous night of dosing i.e. (overnight) fasting before dosing. The drinking water was given *ad libitum* in polypropylene bottles with stainless steel sipper tube.

2.4 Preparation of Test formulation for administration:

1. Test drug **:** Pippalyadi yoga

2. Vehicle **:** Gum acacia

3. Dose preparation **:** The test formulation supplied by the sponsor was made in to fine suspension in vehicle with suitable concentration.

All the animals were dosed constant dose volume (1 ml/ 100g body weight) 175mg/kg, 550mg/kg, 2000mg/kg

4. Schedule **:** Single dose per animal

a) Administration **:** The test formulation was administered through oral route at

different dose levels to respective animal through oral feeding needle on to disposable syringe

b) Dose fixation **:** According to the AOT Software.

C) Route **:** Oral

d) Dose **:** 175mg/kg, 550mg/kg, 2000mg/kg test substance

e) Dose volume **:** 1ml/100g animal

3. Observation:

3.1 Examination of Physical and Behavioral changes :

The animals were observed continuously for 4 hours after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of the every hour the animal was individually exposed to open arena for recording the behavioral changes like increased or decreased motor activity , convulsions, Straub's reaction, muscle spasm, catatonia, spasticity, opisthotonos , hyperesthesia , muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation , diarrhea, writhing , mode of respiration, changes in skin color etc. exitus, CNS depression – hypo activity, passivity, relaxation, ataxia, narcosis, etc.

3.2 Mortality:

All the animals were observed at ½, 1, 2, 3, 4, 24 h, 48 h after dosing and there after daily once for mortality during the entire period of the study (i.e.,14 days).

METHODOLOGY OF OBJECTIVE 3

METHOLOGY OF EFFICACY STUDY METHODS

MATERIALS AND METHODS:

a. Test drug: Pippalyadi yoga (Trikatu and Nagakeshara curna) taken as test drug to be given internally.

Dose selection: Dose fixation: Based on the body surface area ratio by referring to the table of Paget and Barnes (1964)(Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics. Vol. 1. New York: Academic Press; 1964. p. 161.).

Dose for rats:

Pippalyadi yoga was taken from SDM TEACHING PHARMACY,HASSAN.

Human dose X 0.018 for rat weighing 200g

i.e., 12g X 0.018 X 5 = 1.8 gm/kg

i.e.,1g = 0.0018 gm.

Route of drug administration: The drug was administered by oral route with the help of feeding tube attached to injection syringe.

Duration of study: 21 days

b. **THE ANIMALS :**

Wistar strain albino female rats weighing between 180-250g was used for experimental study with following conditions.

The animals were obtained from the animal house attached to the Pharmacology Laboratory, SDM Centre for Research in Ayurveda and Allied Science, Udyavara.

They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature, humidity. They were fed with rat pellets and tap water *ad libitum*

The experiments would be carried out in conformity with guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining its permission.

METHOD:-

The selected animals were grouped into 6 groups with 6 animals each

Animal grouping:

Sl.No	GROUP		Rats
1	**Normal control**	Water control	**6**
2	**Positive control**	Norethisterone	**6**
3	**Test Group I**	Pippalyadi Yoga with Ghritha	**6**
4	**Test Group II**	Pippalyadi Yoga without Ghritha	**6**
5	**Test Group III**	Norethisterone +Pippalyadi Yoga with Ghritha	**6**
6	**Test Group IV**	Norethisterone Pippalyadi Yoga with Ghritha	**6**

Table no 21- Animal Grouping

Drug Administration:

Test drugs were administered for 21 days including experiment day in the morning session between 9-10am orally after taking the cervical smear.

In case of 5th group and 6th group first Norethisterone was administered subcutaneously between 9-10 am after taking the cervical smears then after 1 hour Pippalyadi yoga was administered orally

c. **INCLUSION CRITERIA:**

- Healthy albino female rats only, with average weight of 180-250gms, were selected randomly for the study.
- Rats exposed to Norethisterone preventing ovulation.

d. EXCLUSION CRITERIA:

- Diseased
- Pregnant
- The healthy albino rats which are under other experiments.

e. DRUGS AND CHEMICALS USED:

- Norethisterone from PENTA PHARMACEUTICALS,
- Normal saline
- 10% Formalin
- Hormonal assay kit.

f. DRUG PREPARATION:

For rat dose – norethisterone acetate = 50 ug/10 ml=0.05mg/kg

1 tab of norethisterone acetate=5mg=5000mcg

Stock solution, normal saline- 500 ml

So, dissolve 5ooomcg/5 ml in normal saline= 100 mcg/ml=0.1 ml

g. ROUTE OF ADMINISTRATION:-

The drug was administered by subcutaneous route with the help of needle attached to injection syringe of 0.5ml.

Duration of study: 21 days

h. **OBSERVATION PARAMETER:**

(i) **Ponderal changes:** Body weight, weight of Uterus.

(ii) **In vivo investigations:** Estrous cycle monitoring.

(iii) **Biochemical Parameters:** Serum total cholesterol ,Serum triglyceride, Cholesterol and Triglyceride levels in ovary.

(iv) **Hormonal study:** Serum Estradiol, Progesterone

(v) **Histopathology of** uterus, Ovary, fallopian tube.

i. VAGINAL SMEAR

After grouping the animals , vaginal smear was taken from all the rats from 6 group in the morning 9-10am daily for 21 days including the day of experiment followed by drug administration.

Procedure- A drop of normal saline into the vagina was introduced and with the help of dropper the vaginal secretions were collected and placed on a clean slide. The slides were stained by concentrated solution of Methylene blue and observed under low power microscope.

Assessment of different phases of estrous cycle was done by observing the alterations in the vaginal cells.

j. BLOOD SAMPLE COLLECTION

Blood (2ml) was collected from each animal via retro orbital sinus with heparinized capillary tube and put into plain sample bottle for estrogen progesterone analysis.

k. DISSECTION

After the smear ,21st day, the rats were sacrificed. Over the abdomen of the rats, incision was given after anesthesia. The incision was extended about to the neck and below to the vagina. The uterus, ovary and fallopian tube liver, kidney, heart was excised out from sacrificed animal. The uterus and the ovary were weighed and traffered to the 10% formalin solution for histological examination. The extra deposition from the ovaries were taken out by dissecting the ovaries and weighed. From each rat for the estimation of cholesterol and triglycerides the right vary was processed and sent for histological study.

l. STATISTICAL ANALYSIS

All the values are expressed as MEAN +_ SEM followed by employing one way ANOVA as statistical test followed by Dennett's multiple "t" test as post hoc test. Graph pad inst 3 was used for this purpose. P value < 0.05 is considered as statistically significant. Level of significance was observed noted and interpreted according to the duration of the study for 20 days.

CHAPTER 7

ANALYSIS AND INTERPRETATION

In this chapter the results of objective 1, 2 is included and analysis and result of objective 3 efficacy study is done

RESULTS OF OBJECTIVE 1- PHYTOCHEMICAL STUDIES-

Parameters of Pippalyadi yoga	**Results obtained with n=3%w/w**
Drug lost on drying	9.21 +/- 0.01
Total drug ash	6.19+/-0.22
Ash which was insoluble in acid	0.45+/-0.01
Ash which was soluble in water	3.14+/-0.11
Extractive value of drug soluble in alcohol	13.53+/-0.00
Extractive value of drug soluble in water	23.04+/-0.04

Table 22 (a). Standardization parameters of Pippalyadi yoga

Test	**Inference**
	Pippalyadi yoga
Alkaloid	**+**
Steroid	**+**
Carbohydrate	+
Tannin	+
Flavonoids	+
Saponins	-
Terpenoid	+
Coumarins	+
Phenols	-
Carboxylic acid	-
Amino acids	-
Resin	-
Quinone	-

(+) – present; (-) – negative

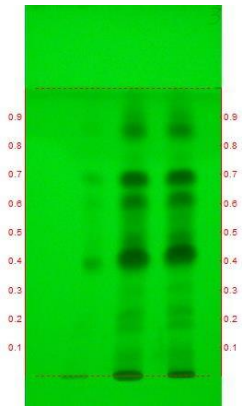
Table 22(b) . Results of preliminary phytochemical screening of Pippalyadi yoga

Tests	Colour if positive	Pippalydi Yoga
Alkaloids		
Dragendroff's test	Orange red precipitate	Orange red precipitate
Wagners test	Reddish brown precipitate	Reddish brown precipitate
Mayers test	Dull white precipitate	Dull white precipitate
Hagers test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann- buchard test	Bluish green colour	Bluish green colour
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and
Carbohydrate		
Molish test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedict's test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Green
Flavonoids		
Shinoda's test	Red or pink	Pink to red

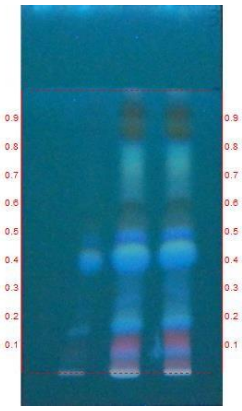
Saponins		
With NaHCO3	Stable froth	No stable froth
Triterpenoids		
Tin and thionyl chloride test	Pink/ Red color	Red color
Coumarins		
With 2 N NaOH	Yellow	Yellow precipitate
Phenols		
With alcoholic ferric chloride	Blue to blue black	Green color
Carboxylic acid		
With water and NaHCO3	Brisk effervescence	No brisk effervescence
Amino acid		
With ninhydrine Reagent	Purple colour	Golden yellow
Resin		
With aqueous Acetone	Turbidity	No turbidity
Quinone		
Conc. Sulphuric acid	Pink/purple/red	Yellow precipitate

Table no 23-Phytochemical test interpretation

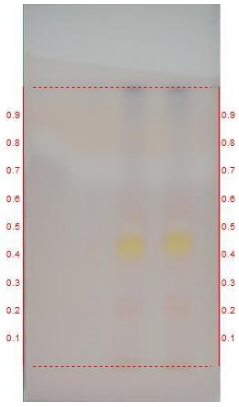
Solvent system: Toluene: Diethyl ether: Ethyl acetate (12.5: 4.3:3.2)



Short UV



Long UV



After derivatization

Figure 34 : HPTLC Photo documentation of sample of Alcoholic extract of Pipallyadi Yoga

Track 1: Alcoholic extract of Pippalyadi yoga- 3µl Track 2: Alcoholic extract of *Pippalyadi yoga*- 6µl Track 3: Alcoholic extract of Pippalyadi yoga- 9

Short UV	Long UV	After Derivatization
-	0.03 (F. red)	-
0.08 (Green)	0.08 (F. purple)	-
-	0.12 (F. pink)	-
0.19 (Green)	0.19 (F.Blue)	-
-	-	0.21 (Pink)
0.23 (Green)	-	-
0.31 (Green)	-	-
-	0.40 (F.Blue)	-
0.42 (D. green)	-	0.42 (Yellow)
-	0.45 (F.green)	0.45 (Yellow)
0.50 (D. green)	0.50 (F.Blue)	-
0.56 (Green)	-	0.56 (D. pink)
-	0.59 (F. black)	-
0.62 (D. green)	-	-
0.70 (D. green)	-	-
0.76 (Green)	-	-
-	0.85 (F. black)	-
0.87 (D. green)	-	-
-	0.93 (F. black)	-

***D – dark; F – fluorescent**

Table 24: Rf values of sample of *Pippalyadi yoga*

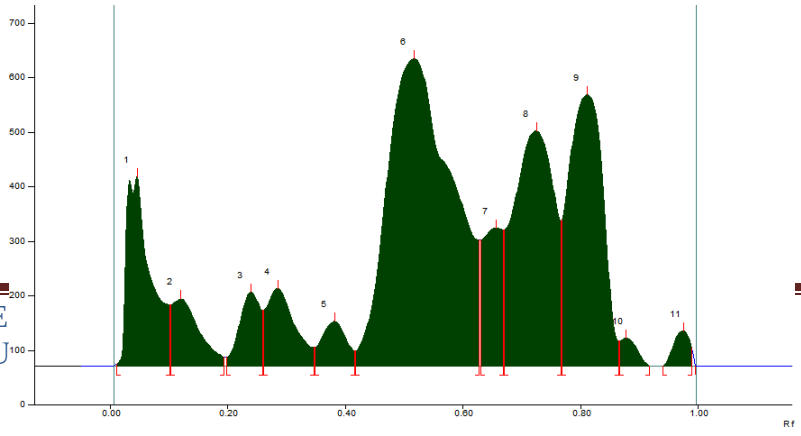
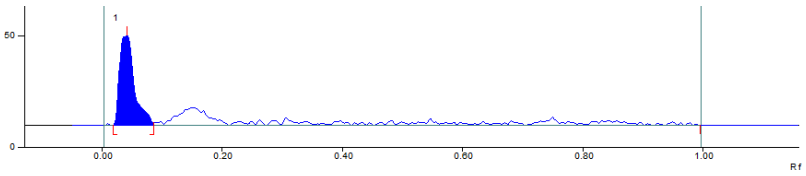


Figure 35: Densitometric scan of the sample of *Pippalyadi yoga*

Track 3, ID: Pippalyadi yoga

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.7 AU	0.05 Rf	347.2 AU	12.90 %	0.10 Rf	12.1 AU	10238.7 AU	8.46 %
2	0.10 Rf	112.1 AU	0.12 Rf	123.4 AU	4.58 %	0.19 Rf	16.9 AU	4192.5 AU	3.47 %
3	0.20 Rf	16.6 AU	0.24 Rf	135.8 AU	5.04 %	0.26 Rf	02.4 AU	3415.3 AU	2.82 %
4	0.26 Rf	102.5 AU	0.29 Rf	141.9 AU	5.27 %	0.35 Rf	33.9 AU	4952.8 AU	4.09 %
5	0.35 Rf	34.3 AU	0.38 Rf	82.1 AU	3.05 %	0.42 Rf	27.9 AU	2479.1 AU	2.05 %
6	0.42 Rf	28.1 AU	0.52 Rf	563.7 AU	20.94 %	0.63 Rf	30.7 AU	44761.5 AU	37.01 %
7	0.63 Rf	231.1 AU	0.66 Rf	252.9 AU	9.39 %	0.67 Rf	50.0 AU	6145.9 AU	5.08 %
8	0.67 Rf	250.2 AU	0.73 Rf	431.0 AU	16.01 %	0.77 Rf	66.1 AU	21433.3 AU	17.72 %
9	0.77 Rf	268.2 AU	0.81 Rf	497.9 AU	18.50 %	0.87 Rf	45.9 AU	21045.8 AU	17.40 %
10	0.87 Rf	46.0 AU	0.88 Rf	50.9 AU	1.89 %	0.92 Rf	0.2 AU	1040.4 AU	0.86 %
11	0.94 Rf	0.4 AU	0.98 Rf	64.9 AU	2.41 %	0.99 Rf	33.0 AU	1254.6 AU	1.04 %

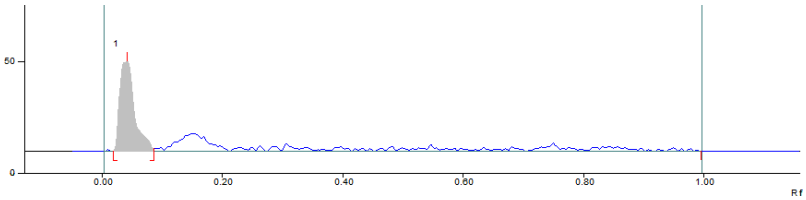
Fig36 (a) At 254nm



Track 3, ID: Pippalyadi yoga

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.04 Rf	40.3 AU	100.00 %	0.09 Rf	1.3 AU	721.7 AU	100.00 %

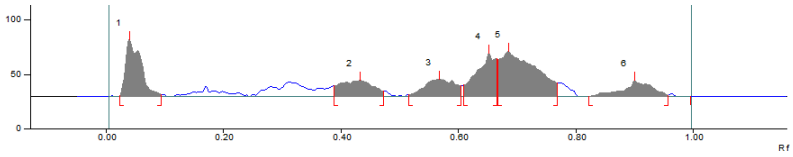
Fig37 (c) At 366nm



Track 3, ID: Pippalyadi yoga

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.04 Rf	40.3 AU	100.00 %	0.09 Rf	1.3 AU	721.7 AU	100.00 %

Fig38 (d) At 540nm



Track 3, ID: Pippalyadi yoga

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.5 AU	0.04 Rf	52.2 AU	29.20 %	0.09 Rf	2.0 AU	978.5 AU	18.08 %
2	0.39 Rf	9.6 AU	0.43 Rf	14.9 AU	8.34 %	0.47 Rf	4.7 AU	601.5 AU	11.11 %
3	0.52 Rf	1.8 AU	0.57 Rf	15.9 AU	8.91 %	0.60 Rf	9.9 AU	597.1 AU	11.03 %
4	0.61 Rf	10.4 AU	0.65 Rf	40.1 AU	22.42 %	0.67 Rf	34.5 AU	902.1 AU	16.67 %
5	0.67 Rf	34.2 AU	0.69 Rf	41.2 AU	23.02 %	0.77 Rf	12.1 AU	1824.8 AU	33.72 %
6	0.82 Rf	0.2 AU	0.90 Rf	14.5 AU	8.09 %	0.96 Rf	1.2 AU	508.3 AU	9.39 %

Fig39 (e) At 620nm

RESULTS OF OBJECTIVE 2 - ACUTE TOXICITY STUDY

Physical and behavioral examination:

There were no physical and behavioral changes-except mild increase in motor activity, irritability ,Piloerection and rearing activity seen in 2 rats in the group 2000mg/kg in all the treated animals on day one at 1,2,3,4 hours intervals after dosing and there after once daily for 14 consecutive days. Thus, the data obtained from the study on single dose administration of coded drug Pippalyadi yoga oral administration up to 14 days of observation period does not result in any physical and behavioral changes.

Mortality:

All the animals belonging to the treated group survived throughout the 14 days observation period after dosing.

Conclusion:

The test drug Pippalyadi yoga did not produce any mortality up to the dose of 2000mg/kg per oral which is equivalent to 22.4g total dose for a human being weighing 70 kg man. At the dose level studied the drug also did not produce any observable toxic effect except for mild irritation in animal in dose550mg/kg and 2000mg/kg and thus it could be concluded that the test drug is without any toxic potential even at the dose of 2000mg/kg in animals equivalent to 22.4g for human being

OBSERVATION AND RESULT OF OBJECTIVE 3-EFFICACY STUDY

EFFECT OF PIPPALYAADI YOGA ON DIFFERENT PHASES OF ESTROUS CYCLE

Pro estrus phase:

Groups		% Change
Normal control	2.66 ±0.55	
Positive control Norethisterone	5.5±1.28*	106.76 ↑@
Pippalyadi yoga with Ghritha (T1)	2.83±0.79	6.39↑@
Pippalyadi yoga without Ghritha(T2)	1.50±0.34	43.60↓@
Norethisterone + Pippalyadi yoga with Ghritha (T3)	1.66 ± 0.33**	69.81↓#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	2.16 ±0.47*	60.72↓#

Data: MEAN ± SEM, * P<0.05, **P<0.01

Table 25: The effect of Pippalyadi yoga on pro estrus phase

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of pro estrous phase in complete Estrous cycle has been shown in the Table 25.

Data shows that there was increase in the total number of proestrous phases in the Norethisterone group compared to normal control group was found to be statistically significant .There was increase in the total number of proestrous phases in the Pippalyadiyoga+Ghritha group compared to normal control group was found to be statistically non -significant .

There is decrease in the total number of pro estrous phases in the Pippalyadi yoga

without Ghritha, which is statistically non-significant when compared to normal control group. Pippalyadi yoga with Ghritha +Norethisterone , Pippalyadi yoga without Ghritha,+Norethisterone , groups were found to be statistically significant when compared to positive control group .

Groups		% Change
Normal control	4.00±1.03	
Positive control (Norethisterone)	4.50±1.28	12.50↑@
Pippalyadi yoga with Ghritha (T1)	4.00±0.44	0 @
Pippalyadi yoga without Ghritha(T2)	4.16±1.01	4↑@
Norethisterone + Pippalyadi yoga with Ghritha (T3)	5.66±0.33	25.77↑#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	3.33±0.84	26↓#

Meta estrus phase

Data: MEAN ± SEM

Table 26: The effect of Pippalyadi yoga on Meta estrus phase

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of Meta estrous phase in complete Estrous cycle has been shown in the Table 26 .

Data shows that there was increase in the total number of metoestrous phases in the Norethisterone group compared to normal control group was found to be statistically non- significant .

There were no any changes has seen in the total number of metoestrous phases in the Pippalyadi yoga with Ghritha group compared to normal control group was found to be statistically non-significant.

There was increase in the total number of metoestrous phases in Norethisterone + Pippalyadi yoga with Ghritha compared to normal control group was found to be statistically non-significant

There was increase in the total number of metoestrous phases in the group Pippalyadi yoga without Ghritha compare to normal control group was found to be statistically non-significant.

Pippalyadi yoga without Ghritha +Norethisterone , groups were found to be statistically non- significant when compared to standard control group as there was decrease in the number of metaestrous phase.

Di estrus phases

Groups		%Change
Normal control	11.5 ±0.95	
Positive control (Norethisterone)	5.66±0.88**	50.78↓@
Pippalyadi yoga with Ghritha (T1)	5.16±0.60**	55.13↓@
Pippalyadi yoga without Ghritha(T2)	5.16±0.87**	55.13↓@
Norethisterone +Pippalyadi yoga with Ghritha(T3)	5.00±0.57	3.10↓#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	3.50±0.42	32.17↓#

Table 27: The effect of Pippalyadi yoga on Di estrus phase

Data: MEAN ± SEM, **P<0.01

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of Di estrous phase in complete Estrous cycle has been shown in the Table 27.

Data shows that there was decrease in the number of Diestrous phase in the Positive control group with Norethisterone in compare with normal control group which is statistically significant.

Data shows that there was decrease in the number of Diestrous phase in the Pippalyadi yoga with Ghritha group in compare with normal control group which is statistically significant.

Data shows that there was decrease in the number of Diestrous phase in the Pippalyadi yoga without Ghritha group in compare with normal control group which is statistically significant.

Data shows that there was decrease in the number of Diestrous phase in the Norethisterone +Pippalyadi yoga with Ghritha in compare with positive control group which is statistically non- significant.

Data shows that there was decrease in the number of Diestrous phase in the Norethisterone + Pippalyadi yoga without Ghritha in compare with positive control group which is statistically non-significant.

Estrus phase

Groups		% Change
Normal control	2.83±0.40	
Positive control (Norethisterone)	1.50±0.22	46.99↓@
Pippalyadi yoga with Ghritha (T1)	6.66±0.88**	135.33↑@
Pippalyadi yoga without Ghritha(T2)	6.33±0.66**	123.67↑@
Norethisterone + Pippalyadi yoga with Ghritha(T3)	5.50±0.42**	266.66↑#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	7.33±0.66**	388.66↑#

Table 28: The effect of Pippalyadi yoga on estrus phase

Data: MEAN ± SEM, **P<0.01

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of estrous phase in complete Estrous cycle has been shown in the Table 28.

Data shows that there was decrease in the total number of estrous phases in the Norethisterone group compared to normal control group was found to be statistically non-significant.

There is increase in the total number of estrous phases in the Pippalyadi yoga with

Ghritha and Pippalyadi yoga without Ghritha group was found to be statistically significant when compared to normal control group..

There was increase in the total number of estrous phases in the Norethisterone + Pippalyadi yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghritha groups was found to be statistically very significant when compared to positive control group .

Serum cholesterol

Groups		% Change
Normal control	46.5±1.52	
Positive control (Norethisterone)	60.5 ±7.86	30.10↑@
Pippalyadi yoga with Ghritha (T1)	41.16±3.09	11.48↓@
Pippalyadi yoga without Ghritha(T2)	50.5±2.95	8.60↑@
Norethisterone + Pippalyadi yoga with Ghritha(T3)	57.0 ±3.55	5.78↓#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	56.5 ±2.91	6.61↓#

Data: MEAN ± SEM

Table 29: The effect of Pippalyadi yoga on serum cholesterol

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of serum cholesterol in complete Estrous cycle has been shown in the Table 29.

Data shows that there was Increase in the level of serum cholesterol in the Norethisterone group compared to normal control group was found to be statistically significant .

There was decrease in the level of serum cholesterol Pippalyadi yoga with Ghritha compared with normal control group was found to be statistically non-significant.

Data shows that there was increase in the level of serum cholesterol in the Pippalyadi yoga without Ghritha compared to normal control group was found to be statistically significant

There was decrease in the level of serum cholesterol in the Norethisterone + Pippalyadi yoga with Ghritha & Norethisterone + Pippalyadi yoga without Ghritha groups compared to positive control group was found to be statistically significant.

Serum Triglycerides

Groups		% Change
Normal control	44.5±4.56	
Positive control (Norethisterone)	85.66 ±2.66	92.49↑@
Pippalyadi yoga with Ghritha(T1)	116.33±4.08**	161.41↑@
Pippalyadi yoga without Ghritha(T2)	133.33±21.23**	199.61↑@
Norethisterone + Pippalyadi yoga with Ghritha (T3)	129.66 ±6.81	51.36↑#
Norethisterone + Pippalyadi yoga without Ghritha (T4)	156.66 ±18.46**	82.88↑#

Table 30: The effect of Pippalyadi yoga Serum Triglycerides

Data: MEAN ± SEM, **P<0.01

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of serum Triglycerides in complete Estrous cycle has been shown in the Table 30.

Data shows that there was increase in the level of serum triglyceride in the Norethisterone group compared to normal control group was found to be statistically non- significant .There was increase in the level of serum triglyceride in Pippalyadi yoga with Ghritha group and Pippalyadi yoga without Ghritha group compared to normal control group were found to be statistically highly significant.

Data shows that there was increase in the level of serum triglyceride in the Norethisterone + Pippalyadi yoga with Ghritha group compared to positive control group was found to be statistically non- significant .

Data shows that there was increase in the level of serum triglyceride in the Norethisterone + Pippalyadi yoga without Ghritha group compared to positive control group was found to be statistically highly significant.

Ovarian cholesterol

Groups		% Change
Normal control	3.83±0.24	
Positive control Norethisterone	4.96±0.54	29.50↑@
Pippalyadi yoga with Ghritha (T1)	4.45±0.14	16.18↑@
Pippalyadi yoga without Ghritha (T2)	4.11±0.30	7.31↑@
Norethisterone + Pippalyadi yoga with Ghritha (T3)	5.13±0.25	3.42↑#
Norethisterone + Pippalyadi yoga without Ghritha (T4)	4.51±0.46	9.07↓#

Data: MEAN ± SEM

Table 31: The effect of Pippalyadi yoga on ovarian cholesterol

Data related to the effect of Pippalyadi yoga on ovarian cholesterol in complete Estrous cycle has been shown in the Table 31.

Data shows that there was increase in the level of cholesterol in ovary of the Norethisterone group, Pippalyadi yoga with Ghritha and Pippalyadi yoga without Ghritha group compared to normal control group was found to be statistically non-significant.

There was increase in the level of cholesterol in ovary of the Pippalyadi yoga with Ghritha + Norethisterone group when compared with positive control group was found to be statistically non-significant.

There was decrease in the level of cholesterol in ovary of the Pippalyadi yoga without Ghritha + Norethisterone group when compared with positive control group was found to be statistically non-significant

Ovarian Triglyceride

Groups		% Change
Normal control	95.66±6.09	
Positive control Norethisterone	45.83±2.52**	52.09↓@
Pippalyadi yoga with Ghritha (T1)	65.5±2.72**	31.52↓@
Pippalyadi yoga without Ghritha (T2)	58.33±9.74**	39.02↓@
Norethisterone + Pippalyadi yoga with Ghritha (T3)	53.83±5.26	17.45↑#
Norethisterone + Pippalyadi yoga without Ghritha (T4)	63.83 ±7.99	39.27↑#

Table 32: The effect of Pippalyadi yoga on ovarian Triglyceride

Data: MEAN ± SEM, **P<0.01

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of ovary TG in complete Estrous cycle has been shown in the Table 32.

Data shows that there was decrease in the level of triglyceride level in the ovary of Norethisterone group was found to be statistically significant when compared to normal control group. Data shows that there was decrease in the level of triglyceride in the ovary of Pippalyadi yoga with Ghritha and Pippalyadi yoga without Ghritha group was found to be statistically highly significant when compare to normal control group. There was an increase in the level of triglyceride in the ovary of Norethisterone + Pippalyadi yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghritha groups was found to be statistically non- significant when compared to positive control group .

Wt. of Uterus

Groups		% Change
Normal control	4.06±0.25	
Positive control (Norethisterone)	3.95±0.51	2.70↓@
Pippalyadi yoga with Ghritha (T1)	4.43±0.43	9.11↑@
Pippalyadi yoga without Ghritha(T2)	4.03±0.25	0.73↑@
Norethisterone + Pippalyadi yoga with Ghritha(T3)	4.35±0.23	10.12↑#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	3.75±0.25	5.06↓#

Data: MEAN ± SEM

Table 33: The effect of Pippalyadi yoga on Wt. of Uterus

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of Wt. of Uterus in complete Estrous cycle has been shown in the Table 33. In positive control group with Norethisterone compared to normal control group there is statistically non – significant decrease in the weight of uterus noted.

In Pippalyadi yoga with Ghritha group compared to normal control group there is statistically non –significant decrease in the weight of uterus noted.

Pippalyadi yoga without Ghrita, Norethisterone + Pippalyadi yoga with Ghrita, Norethisterone + Pippalyadi yoga without Ghrita groups compared to positive control group there is statistically non –significant increase in the weight of uterus noted.

Body weight

Groups		% Change
Normal control	39.90 ±5.54	
Positive control Norethisterone	22.87±3.31	42.68↓@
Pippalyadi yoga with Ghritha (T1)	23.77±6.16	40.42↓@
Pippalyadi yoga without Ghritha(T2)	30.41±9.92	23.78↓@
Norethisterone + Pippalyadi yoga with Ghritha(T3)	14.77±4.10	35.41↓#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	14.23±4.70	37.77↓#

Table 34: The effect of Pippalyadi yoga on % change in body wt.

Data: MEAN ± SEM , @-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of % change in body wt. in complete Estrous cycle has been shown in the Table 34.

Here in all groups statistically non -significant decrease in weight was seen. In positive control group with Norethisterone compared to normal control group there is statistically non –significant decrease in the weight of body noted. In Pippalyadi yoga with Ghritha group compared to normal control group there is statistically non –significant decrease in the weight of body noted. Pippalyadi yoga without Ghrita, Norethisterone+Pippalyadi yoga with Ghrita, Norethisterone + Pippalyadi yoga without Ghrita groups compared to positive control group there is statistically non –significant increase in the weight of uterus noted.

Serum Estradiol

Groups		% Change
Normal control	36.66 ±4.07	
Positive control (Norethisterone)	33.00±0.63	9.98↓@
Pippalyadi yoga with Ghritha (T1)	34.16±3.51	6.81↓@
Pippalyadi yoga without Ghritha(T2)	46.66±1.30	27.27↑@
Norethisterone + Pippalyadi yoga with Ghritha(T3)	40.33±1.14	22.21↑#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	36.66±4.07	11.09↑#

Table no. 35: The effect of Pippalyadi yoga on Serum Estradiol

Data: MEAN ± SEM

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of serum Estradiol in complete

Estrous cycle has been shown in the Table no. 35

Data shows that there was decrease in level of serum estrogen in the Norethisterone group and Pippalyadi yoga with Ghrita group was found to be statistically non- significant compared to normal control group.

In groups Pippalyadi yoga without Ghrita ,Norethisterone + Pippalyadi yoga with Ghrita and Norethisterone + Pippalyadi yoga without Ghrita there was statistically non-significant increase in the level of serum estradiol was observed when compared to positive control group.

Serum progesterone

Groups		% Change
Normal control	18.53±1.98	
Positive control (Norethisterone)	11.23±0.66*	39.39↓@
Pippalyadi yoga with Ghritha (T1)	11.55±0.46*	37.66↓@
Pippalyadi yoga without Ghritha(T2)	10.3±1.08**	44.41↓@
Norethisterone + Pippalyadi yoga with Ghritha(T3)	11.08±0.68	40.20↓#
Norethisterone + Pippalyadi yoga without Ghritha(T4)	15.53 ± 2.87	16.18↓#

Table no. 36: The effect of Pippalyadi yoga on serum Progesterone

Data: MEAN ± SEM, * P<0.05, **P<0.01

@-compared with normal control #-compared with Positive control

Data related to the effect of Pippalyadi yoga on no. of serum progesterone in complete Estrous cycle has been shown in the Table 36.

The data shows there was statistically decrease in serum progesterone in Norethisterone group, Pippalyadi yoga with Ghrita , groups when compared to normal control group.

In Pippalyadi yoga without Ghrita group there was statistically highly significant decrease in the serum progesterone was seen when compared with normal control group. In groups with Norethisterone + Pippalyadi Yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghrita there was statistically non –significant decrease in serum progesterone noted when compared to positive control group.

Changes in the Uterus

Control group

Rat no And section	**Changes observed**
1	Atrophic epithelium. No eosinophils. No sub nuclear vacuoles in epithelium and glands
2	Atrophied endometrium. Decreased number of glands. Stroma loosely arranged.
3	Thickened epithelium and myometrium. Proliferation o

Table no.37-Changes in the uterus in control drug

NORETHISTERONE

Rat no and section	**Changes observed**
1	Epithelium hyperplastic with many cell layers. Numerous degenerated vacuoles seen in epithelium and glands. Many vacuoles are seen in epithelium. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Many congested blood vessels seen in myometrium.
2	Epithelium hyperplastic with many cell layers. Numerous degenerated vacuoles seen in epithelium and glands. Many vacuoles are seen in epithelium. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Many congested blood vessels seen in myometrium
3	Epithelium hyperplastic with many cell layers. Numerous degenerated vacuoles seen in epithelium and glands. Many vacuoles are seen in epithelium. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Many congested blood vessels seen in myometrium

Table no 38- Changes in Uterus in Norethisterone drug administration

T1 Pippalyadi yoga with Ghrita

Rat no and section	**Changes observed**
1	Thickened epithelium. More glands compared with Ctl. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation Proliferating blood vessels seen in endometrium and myometrium. Inflammatory infiltrated seen in 1 area. Vacuoles with secretary material seen in glands and epithelium. Cystic changes of glands seen.
2	Thickened epithelium. More glands compared with Ctl. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation Proliferating blood vessels seen in endometrium and myometrium Vacuoles with secretary material seen in glands and epithelium
3	Thickened epithelium. .More glands compared with Ctl. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation Proliferating blood vessels seen in endometrium and myometrium. Vacuoles with secretary material seen in glands and epithelium

Table no.39- T1 Pippalyadi yoga with Ghrita

T2 Pippalyadi yoga without Ghrita

Rat no and Section	Changes observed
1	Many vacuoles with secretory materials are seen in epithelium. More glands compared with Ctl. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Proliferating blood vessels seen in endometrium and Myometrium.
2	Many vacuoles with secretory materials are seen in epithelium. More glands compared with Ctl. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Proliferating blood vessels seen in endometrium and myometrium. Cystic change of glands seen
3	More glands compared with Ctl. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Proliferating blood vessels seen in endometrium and myometrium

Table no.40- T2 Pippalyadi yoga without Ghrita

Norethisterone + Pippalyadi yoga with Ghrita T3

Rat no and section	**Changes observed**
1	There is reduction of vacuoles, and eosinophil There is no epithelial hyperplasia. Cellular and stromal proliferation. Increased glands seen.
2	There is reduction of vacuoles, and eosinophil There is no epithelial hyperplasia. Cellular and stromal proliferation. Increased glands seen
3	There is reduction of vacuoles, and eosinophil There is no epithelial hyperplasia. Cellular and stromal proliferation. Increased glands seen

Table no. 41- Norethisterone + Pippalyadi yoga with Ghrita T3

Norethisterone + Pippalyadi yoga without Ghrita(T4)

Rat no and section	Changes observed
1	Epithelium atrophic. . Proliferation of stroma and hypercellular layers. Gland damage seen in some areas.
2	Epithelium atrophic. . Proliferation of stroma and hypercellular layers. Gland damage and hyperplasia seen in some areas
3	Epithelium atrophic. Proliferation of stroma and hypercellular layers. Gland damage seen in some areas.

Table no 42- Norethisterone + Pippalyadi yoga without Ghrita(t4)

Weight of ovary in control group

Rat no and section	**Changes observed**
1	Very few degenerating follicles. Many viable follicles.
2	Many viable follicles. Very few degenerating follicle
3	Many viable follicles. Very few degenerating follicle

Table no 43- Weight of Ovary in control group

Weight of Ovary in Norethisterone group

Rat no and section	**Changes observed**
1	More degenerating follicles. More corpus luteum.
2	Increase number of follicles. But most of them are degenerating. More corpus Luteum
3	Very less follicles. Nearly all are degenerating. Congested blood vessels.

Table no 44-weight of ovary in Norethisterone group

T1 Pippalyadi yoga with Ghrita

Rat no and section	Changes observed
1	Very less viable follicles, more degenerating follicles compared with control. Many congested blood vessels are also seen.
2	Very less viable follicles (1 follicle), more degenerating follicles compared with control. Many congested blood vessels are also seen. Ovary is atrophic
3	Very less follicle. No viable follicle. More corpus luteum seen

Table no 45- T1 Pippalyadi yoga with Ghrita

T2 Pippalyadi yoga without Ghrita

Rat no and section	Changes observed
1	Many degenerating follicles. Very few viable follicles
2	Many degenerating follicles. Very few viable follicles
3	Many degenerating follicles. Very few viable follicles

Table no 46- T2 Pippalyadi yoga without Ghrita

Norethisterone + Pippalyadi yoga with Ghrita T3

Rat no and section	Changes observed
1	More follicles and less degenerating follicles seen. Few congested blood vessels seen.
2	Very few follicles and more degenerating follicles
3	More follicles. Corpus luteum showed many vacuoles. Few congested blood vessels seen.

Table no 47- Norethisterone + Pippalyadi yoga with Ghrita T3

Norethisterone + Pippalyadi yoga without Ghrita T4

Rat no and section	Changes observed
1	More follicles and less degenerating follicles seen
2	Many degenerating follicles. Very few viable follicles
3	Less number of follicles.

Table no 48- Norethisterone + Pippalyadi yoga without Ghrita T4

Consolidated report of Changes in Uterus and ovaries.

Groups	Uterus	Ovary
CONTROL GROUP	The sections showed atrophied epithelium with loosely arranged stroma.	Many viable follicles with very few degenerating follicles. Few interstitial glands seen
NORETHISTERONE GROUP	Numerous degenerated vacuoles seen in epithelium and glands. Many vacuoles are seen in epithelium. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Many congested blood vessels seen in myometrium	Reduced follicles. More degenerating follicles. carpus luteum
T1	All sections showed thickened epithelium. with more glands. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation Proliferating blood vessels seen in endometrium and myometrium. Vacuoles with secretary material	Follicles are very less. Moderate degenerating follicle seen. congested blood vessels are seen

	seen in glands and epithelium	
T2	The sections showed moderate increased epithelium with vacuoles containing secretory materials. Abundance of eosinophils and blood vessels seen very few all layers.-secretory endometrium	Very few viable follicles seen. Mild degenerating follicles seen.
Norethisterone +T3	There is reduction of vacuoles, and eosinophil There is no epithelial hyperplasia. Cellular and stromal proliferation. Increased glands seen.	Very few viable follicles seen. Mild degenerating follicles seen
Norethisterone +T4	There is mild reduction of vacuoles, and eosinophils. The rat sections showed severe proliferation of stroma and hypercellular muscular layer.. Moderate increased glands seen.	Increase in number of follicles and viable follicles

Table no 49- report of changes in ovaries

INTERPRETATION OF THE EFFICACY STUDY

Infertility is a problem related to inability of conception due to various causes. Ovarian dysfunction is one among them. Anovulation is a condition which explains the failure of ovulation due to the dysfunction of hypothalamus-pituitary-ovarian axis.

Artava is considered as Stri Beeja (ovum) as far as the conception is concern and Nastartva, the condition without Artava can be considered as anovulation. Nastartava is one type of Artava vikara the result of which is responsible for Asamarthana of Prajotpadana i.e., Vandyatwa. Clinically anovulation is treated with different ovulation induction drugs.

The side effects like ovarian hyper stimulation, early pregnancy loss, , multiple pregnancies of conventionally practiced ovulation induction drugs necessitate the need of other alternate safe treatment with no side effects.

For the management of Vandyatwa there are good numbers of formulations and treatment modalities mentioned in Ayurvedic classics and are well tested, tried and trusted. Pippalyadi Yoga is one such combination with Trikatu and Nagakeshara Churna in equal quantity to be consumed with Ghrita mentioned in Bhaishajya Ratnavali Yonichikitsa for the treatment of Vandyatwa. Hence this combination was taken for analyzing its fertility effect on Primolut -N induced anovulation which was supported by biochemical and histopathological parameters.

Considering the short estrus cycle of albino rats this experimental study with title "Assessment of efficacy of Pippalyadi yoga in the management of Norethisterone induced anovulation in albino rats" was planned.

As the drug diethylstilbestrol is banned in the market, Norethisterone with similar action in inducing anovulation was substituted and change of drug certificate is enclosed.

Phytochemical analysis of the drug showed the presence of alkaloids, carbohydrate, coumarins, flavonoids, phenols, steroids and tannins.

Acute oral toxicity study of Pippalyadi yoga-

The data obtained from the study on single dose administration of coded drug Pippalyadi yoga oral administration up to 14 days of observation period does not result in any physical and behavioral changes. In the toxicity study, toxic symptoms and mortality was studied for the trial drug which was well tolerated up to 2g/kg. No any mortality and toxicity were observed in the rats. The test drug Pippalyadi yoga did not produce any mortality up to the dose of 2000mg/kg per oral which is equivalent to 22.4g total dose for a human being weighing 70 kg man. At the dose level studied the drug also did not produce any observable toxic effect except for mild irritation in animal in dose550mg/kg and 2000mg/kg and thus it could be concluded that the test drug is without any toxic potential even at the dose of 2000mg/kg in animals equivalent to 22.4g for human being.

DISCUSSION ON ESTRUS CYCLE

The reproductive functions are under the regulation of hypothalamus pituitary ovarian axis. The integrity of the reproductive system depends on estrogen and progesterone. The frequency and duration of estrous cycle is different in different organisms.

The length of estrous cycle in rats with 4 to 5 day cycle

1	PROESTROUS	12-14 hrs.
2	ESTROUS	25-27 hrs.
3	METAESTROUS	6-8 hrs.
4	DIESTROUS	55-57 hrs.

Table no 50- Estrus cycle phases in Albino Rats

Assessment of estrus cycle in experimental animal is a useful measure of the integrity of the Hypothalamus-pituitary ovarian axis. It can also be used to investigate the effects of drugs and chemicals on reproductive function frequently expressed as a disruption in the typical morphology, cytology and histology of reproductive organs and alteration in duration of particular phases of the estrus cycle.

Techniques for estrus cycle assessment

Various methods include visual assessment, vaginal cytology, histological examination of the reproductive organs, vaginal wall impedance, urine biochemistry is used to evaluate the estrous cycle based on the changes in the animal's anatomy and physiology.

Among them, the most common technique is vaginal cytology which is widely accepted. It is non-invasive and inexpensive method of evaluation.

The vaginal secretion is made up of three types of cells – leucocytes, corneal epithelial cells and nucleated epithelial cells. Estimation of phase of estrous cycle is based on the proportion of these cells in the vaginal secretion

During assessment the rats were restrained and tail was elevated to visualize the vagina. The vaginal cells were flushed by gently introducing distilled water with pipette. Slowly released the liquids into the vagina and retracted back. This was repeated 4 to 5 times. After that strain was introduced.

Norethisterone

Hydroxy-norprogestene. These possess a high degree of biological activity which has been demonstrated by animal and experimental studies. Progestins affects the concentrations of hormones in the serum particularly estrogen. They are capable of inhibiting the folliculogenesis by negatively affecting the secretion of hypothalamus and causes anovulation. They produce anti-proliferative changes in the endometrium. As the serum progestin is withdrawn after the second half of the menstrual cycle, menstruation may occur.

DISCUSSION ON THE RESULTS

CONSOLIDATED STATEMENT OF ESTROUS CYCLE

PARAMETERS	**COMPARED WITH NORMAL.**			**COMPARED WITH NORETHISTERONE**	
	Norethisterone	T1	T2	T3	T4
PROESTROUS	SI	NSI	NSD	SD	SD
ESTROUS	NSD	SI	SI	SI	SI
METAESTROUS	NSI	NC	NSI	NSI	NSD
DIESTROUS	SD	SD	SD	NSD	NSD

Table no 51- Consolidated statement of Estrus cycle

Discussion on different phases of estrus cycle

PROESTROUS PHASE-

The data shows that Proestrous phase was significantly increased in Norethisterone group, compared to normal control group which is needed for the anovulation to occur. In Pippalyadi yoga without Ghritha group, there is non-significant decrease of the proestrous phase as compared to normal control group indicates that test drug is having more estrogenic action and hence responsible for proper vaginal proliferation when it was given without adding Ghritha. Whereas in Norethisterone + Pippalyadiyoga with Ghritha and Norethisterone + Pippalyadiyoga without Ghritha there significant decrease of the Proestrous phases as compared to positive control group.

Sohrabvand et.all[64] state that anti-estrogenic drugs such as clomiphene citrate are the first line of therapy for ovulation induction in the patients with anovulation and are capable in producing ovulation in 70-75% of cases. By this it can infer that the drug may be having the antiestrogenic activity which causes

negative feedback, thereby increasing the F.S.H and L.H to induce follicular development and ovulation.

ESTRUS PHASE –

In Norethisterone group estrous phase was non-significantly decreased compared with normal control group. In Pippalyadi Yoga with Ghritha and Pippalyadi Yoga without Ghritha group there was statistically significant increase in estrus cycle when compared with normal control group.

Norethisterone +Pippalyadi Yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghritha group there was significant increase of estrous phases as compared to positive control group. This indicates there was increased estrogenic activity. Estrogen secreted during the folliculogenesis or by matured secondary follicle might have produced the estrogen hormone and exhibited the estrogenic activity .These changes will promote the ovulation. The estrogen is essential for the preparation of endometrial thickness. The structural changes observed in the vaginal epithelium of female rats during the estrous cycle are induced by estrogen and progesterone. Thus, the rat vagina can be considered a mirror of ovarian function that reflects the activity of sex hormones (Houssay et al., 1951)[65].

METAESTROUS PHASE–

The data shows that in metaestrous phase there was non- significant increase in Norethisterone group as compared to normal control group. In Pippalyadi yoga with Ghritha group no change was observed when compared with normal control group. In Pippalyadi yoga without Ghritha non-significant increase was there compared to normal control group and in Pippalyadi yoga with Ghritha + Norethisterone group showed non-significant increase of metaestrous phases as compared to positive control group. In Norethisterone + Pippalyadi yoga without Ghritha non -significant decrease in metaestrus phase was seen. This shows that there was less availability of secondary Graffian follicles indicating post ovulation decline of hormones. Though the increase in the metaestrous phase was statistically non-significant, but the increase which was observed during the study suggests that there was ovulation.

DIESTROUS PHASE-

The data shows that, in Norethisterone group, Pippalyadi Yoga with Ghritha and Pippalyadi Yoga without Ghritha groups there was significant decrease in diestrus phase when compared with normal control group suggests irregularity of periods. In Norethisterone + Pippalyadi Yoga with Ghritha and Norethisterone + Pippalyadi Yoga without Ghritha groups diestrous phase showed non- significant decrease in comparison with positive control group which indicates restoration of normal estrous cycle.

DISCUSSION ON BIOCHEMICAL CHANGES

Parameters	**Compared with normal**			**Compared with** NORETHISTERONE	
	Norethisterone	T1	T2	T3	T4
Serum cholesterol	NSI	NSD	NSI	NSD	NSD
Serum Triglyceride	NSI	SI	SI	NSI	SI
Ovarian Cholesterol	NSI	NSI	NSI	NSI	NSD
Ovarian Triglyceride	SD	SD	SD	NSI	NSI
Serum Estradiol	NSD	NSD	NSI	NSI	NSI
Serum Progesterone	SD	SD	SD	NSD	NSD

Table no 52- Consolidated statement of Biochemical changes

CHOLESTEROL

Cholesterol is the basic building block in steroidogenesis. Steroid-producing

organs can synthesize cholesterol from acetate. From the 2-carbon acetate molecule Progestins, androgens and estrogens can be synthesized in the various ovarian tissue via cholesterol.[66]

Serum cholesterol- The data shows that, In Pippalyadi yoga with Ghritha group, there is non-significant increase in the serum cholesterol level as compared to normal control group which indicates increased steroidogenesis. In Norethisterone+ Pippalyadi yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghritha group, there is non- significant decrease in the serum cholesterol level as compared to positive control group shows normal steroidogenesis indicating estrogenic activity of the test drug.

Serum triglyceride: The data shows that, In Pippalyadi yoga with Ghritha, Pippalyadi yoga without Ghritha shows significant increase in serum triglyceride compared to normal control group. Norethisterone + Pippalyadi yoga without Ghritha group, there was significant increase in the serum triglyceride level as compared to positive control group which indicates increased steroidogenesis suggesting estrogenic action of test drug .

Ovarian cholesterol- In Pippalyadi yoga with Ghritha group and Pippalyadi yoga without Ghritha group there is non–significant increase in the ovarian cholesterol level as compared to normal control group suggests normal steroidogenesis and drug has estrogenic activity. In Norethisterone + Pippalyadi yoga without Ghritha group, there was non-significant decrease in the ovarian cholesterol level as compared to positive control group indicates that more utilization of the cholesterol for production of estrogen which is responsible for ovulation.

Ovarian triglyceride –The data shows that, In Norethisterone + Pippalyadi yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghritha group there was non–significant increase in the ovarian triglyceride level as compared to positive control group which suggests that adequate amount of estrogen is present for formation of follicles and regularizes ovulation.

Serum cholesterol, ovarian cholesterol, triglyceride and ovarian triglycerides in

Norethisterone +Pippalyadi yoga with Ghritha there was non-significant increase seen.

Vatsala Sivakumar et all[67] .states that ingredients of Trikatu decrease the triglycerides and LDL cholesterol. There by it reduces the risk of hyperlipidemia.

Hormones have important roles in the estrous cycle. The follicular stimulating hormone and luteinizing hormone regulate the estrous cycle. Due to these hormones, there will be follicular changes in the ovary and also changes are evident in vaginal cells. Also. Growth of the follicle is under the stimulation of FSH and the ovulation after luteinization of mature graafian follicle and formation of corpus lutetium is due to the action of LH. The corpus lutetium secrets the progesterone during Meta estrous phase and its level decreases during Di estrous phase. As the follicular growth is active the level of estradiol-17 β also increases in the serum. At the end of estrous cycle, the estrogen level will be peak and starts the Pro estrous cycle which induces ovulation. (Freeman, 1988).

ESTROGEN – Estrogen receptor α and estrogen receptor β are the two receptors of estrogen. estradiol is the important and active estrogen produced by the ovary. As the follicles are maturing granulosa and theca cells of ovary produce estradiol in increased amount. Production of estradiol will be moderate in the beginning and will be at its peak just prior to ovulation. After ovulation its level falls until the corpus luteum is formed. There after as the corpus luteum degenerates in the absence of fertilization its level completely decreases.[68a.] Estrogen is the main hormone responsible for the proliferation of endometrium, growth of uterus and fallopian tubes and vagina. They also bring changes like stratification, cornification and epithelial thickness of the vagina

In the Pippalyadi yoga with Ghritha group , there is non-significant decrease in the serum estrogen level as compared to normal control group shows that there is no any alteration seen in hormonal level and it won't disrupt normal physiology but has very mild estrogenic activity. In the Primolut+ Pippalyadi yoga with Ghrita and Norethisterone + Pippalyadi yoga without group, there is

statistically non -significant increase in the serum estrogen. The metabolism of estrogens takes place in the liver and metabolites are excreted through the urine. If the liver function is impaired active estrogen in circulation increased.it leads to ovulatory dysfunctions.[68.b] So we can say that the drugs in Pippalyadi yoga as they having Yakrututtejaka properties and they help in the proper functioning of liver and estrogen is metabolized normally. Hence its level is not increased in the present study.

PROGESTERONE: - Metabolism of Progesterone happens in the liver. The production of Progesterone during early follicular phase is negligible as it is secreted mainly by corpus luteum. Luteinizing hormone initiates luteinization and progesterone production in the granulosa layer. This preovulatory rise in progesterone promote the positive feedback action of estrogen.[69]

In this experimental study data shows that, in Norethisterone + Pippalyadi yoga with Ghritha and Norethisterone + Pippalyadi yoga without Ghritha group there was non-significant decrease in serum progesterone as compared to positive control group indicates test drug has promoted the ovulation and hence there was maintenance of serum progesterone level.

DISCUSSION ON CHANGES IN WEIGHT.

Parameters	**Compared with normal**			**Compared with NORETHISTERONE**	
	Norethisterone	T1	T2	T3	T4
Weight of uterus	NSD	NSI	NSI	NSI	NSD
Weight of ovary	NSI	SI	SI	NSI	SI

Table no 53- Consolidated statement of changes in weight.

On weight of Uterus: In the Norethisterone + Pippalyadi yoga with Ghritha there was non-significant increase in the weight of the uterus and ovary. Pippalyadi yoga with Ghritha and Pippalyadi yoga without Ghritha group there was non-significant increase in the weight of uterus. Though this increase is statistically non–significant but still slight increase in weight of uterus is observed when compared with positive control group.

On weight of Ovary- The weight of the depends on glands and stromal tissues, follicles and corpus luteum. Due to the action of gonadotrophins and steroidal hormones the weight of the ovarian tissues increases.

In Pippalyadi yoga with Ghritha and Pippalyadi yoga without Ghritha groups shows significant increase in weight of ovary when compared to normal control group. In Norethisterone+ Pippalyadi Yoga without Ghritha groups there was significant increase of weight of ovary noted compared to positive control group suggests the maturation of follicles which releases the hormones which are responsible for the increase in the weight of the tissues. By this we can infer that there was ovulation induction like action by the drug.

Discussion on histology of uterus and ovary

Discussion on histology of uterus

T1— Thickened epithelium. More glands compared with control group.

Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation seen. Proliferating blood vessels seen in endometrium and myometrium. Inflammatory infiltrated seen in 1 area. Vacuoles with secretary material seen in glands and epithelium. Cystic changes of glands seen

This indicates that the test drug has estrogenic activity and increasing the thickness of endometrium by nourishing the endometrial layer. Also indicate the steroidogenesis

T2- Many vacuoles with secretory materials are seen in epithelium. More glands compared with Control. Abundance of eosinophils seen in all layers. Severe cellular and stromal proliferation. Proliferating blood vessels seen in endometrium and myometrium

NORETHISTERONE +T3: There is reduction of vacuoles and eosinophil, there is no epithelial hyperplasia. Cellular and stromal proliferation. Increased glands seen.

NORETHISTERONE +T4: Epithelium atrophic. Proliferation of stroma and hyper cellular layers. Gland damage seen in some areas.

Discussion histology of ovary

T1- Very less viable follicles, more degenerating follicles compared with control. Many congested blood vessels are also seen. More corpus luteum seen.

T2: Many degenerating follicles. Very few viable follicles.

NORETHISTERONE + T3: More follicles and less degenerating follicles seen. Few congested blood vessels seen, Corpus luteum showed many vacuoles.

NORETHISTERONE + T4: More follicles and less degenerating follicles seen.

By this it is evident that the test drug showing ovulation induction action generating the production of steroidal hormones.

ANALYSIS AND INTERPRETATION ACC. TO AYURVEDA

Ayurveda considers menstrual cycle as Artava chakra or Rutuchakra. The menstrual cycle in albino rats is considered as estrus cycle. Artava chakra begins at the age of 12

and ends at 50 in humans.

In rats Rutu chakra starts at 30th day after birth.

Tridosha play important role in Artava chakra. Vata is responsible for normal flow and Pitta governs the secretory Phase or Rutuvyateeta kaala and Kapha has important role during Rutu kaala or ovulatory phase.

This in Albino rats also we can understand according dosha .

Proestrus phase	Rajah kaala and early Rutu kaala	Pitta – Kapha
Estrus phase	Rutukaala	Kapha
Meta estrus phase	Rutu vyatheeta kala	Pitta- Vata
Diestrus phase		Vata

Table no 54- Intrepretation of Estrus cycle in Ayurveda

During Kapha dominated Estrus cycle there will be ovulation which we can considered as beeja rupi Artava. Hence this period is the heat period in rats and fertilization occurs during this phase.

Artava can be considered as Ovum, menstrual blood and Hormones according to the context where the word Artava is used.

In this experimental study the test groups rats were given Norethisterone to induce anovulation.

As this is a chemical, we can consider it as Dushi visha. So, there will be Agni dusti in the rats. Hence causing Rasadhatu dusti leading to Artava Naasha.As a treatment we have given Pippalyadi yoga which contains Trikatu and Nagakeshara curna along with Ghrita as Anupana.

The effect of the treatment was assessed using changes in the vaginal cell which was taken by vaginal smear. During Rutukala in Rutumati laxana it is told by Acharyas that the female will have urge for sexual life. In rats also Estrus cycle is taken as the special period for sexual desire for female.

ASSESSMENT OF EFFECT ON MEDO DHATU

Medodusti due to Santarpana ahara vihara leads to many clinical entities, Due to the Sangha in the Medovaha Srotas, Uttarotara Dhatu Poshana Hampers and affects negatively in the formation of end Dhatu – Shukra/Artava

In this animal study the group with Pipplyaadi yoga without Ghritha showed statistically significant decrease in Medodhathu (Serum cholesterol). Though in Pipplayadi Yoga with Ghritha there was no decrease in the Medodhatu but there was Non-significant Increase observed. Hence, we can say that combination helps in the metabolism of Medo Dhatu Pachana.

ASSESSMENT OF EFFECT ON DHATU RUPI ARTHAVA

Estrodiol- Arthava can be taken as female harmones and liver is taken as the Agni Sthana, the estrogen metabolizes in the liver likewise the Arthava forming Ama in the rasa gets Pachana in the Yakrut due to the action of Pachakagni helping the maintenance of Arthava in the serum

Hence in this study due to Yakrut-Uttejaka Karma of the Yoga in the study group the estrogen level is not increased but maintained.

Progesterone- Progesterone can be considered as the Arthava which is formed at the level of Dimba after the Beejarupi Arthava(ovum) is formed. In Nastarthava condition Beejarupi Arthava formation is affected and hence in the serum the level of Arthava is decreased.

In this study group the Gunas like Katu Rasa, Katu Vipaka and Ushna Veerya of Pippalyadi Yoga by clearing the sangha and helps in the Beeja Rupi Arthava Utpatti in the Dimba. Hence the Arthava(progesterone) level in the serum is increase.

ASSESSMENT ON GARBHASHAYA BALYA EFFECT (WEIGHT OF UTERUS)

The drugs in the yoga are having Rasayana effect and Arthava Janaka Karma which are helps in Garbhashaya Drudeekarana and Balya. In the study group the weight of uterus is statistically increased which implies at the Santarpana effect of the yoga.

DISCUSSION ON INDIVIDUAL DRAVYA-

PIPPALI

Muhammad Asif and Seyedeh Fatemeh j[70] in their mini review on Mesua ferrea state that the Piperine present in the Pippali is known for its bioavailability and it

enhances the digestion. Also, they say that in vitro study showing oxidative stress relieving action of piperine by suppressing free radicals and reactive species.

Neha Choudhary and Vikram Singh state that as Pippali is having bioavailable property it helps the reach of other drugs to reach the target site and initates and promotes the folliculo-genesis.

Ushna Teekshna Guna of Pippali helps in the clearance of Sroto Rodha by its Srotoshodaka property. As Pippali is Rasayana it nourishes the Rasadhatu and promotes the formation of Upadhatu Artava. Neha and Vikram Singh studies show that Pippali improves the brain function due to its neuro modulator property there by regulating the function of HPO axis. The pharmacological properties of this plant also include antioxidant, anti-inflammatory, hepatoprotective which are needed for the normal functioning of the reproductive system.

SHUNTI

Kim, Kang ,Kim, Choung & Zee[71] (2008) analyzed 94 medicinal plants on the yeast cells that were modified to carry the human estrogen receptor (hER gene) and found that out of 14 plants which showed significant effects on the human estrogen receptor. Ginger with concentrations between 0.1 and 2.8 mg/ml were found effective in for producing estrogenic responses. Shekoufeh Atashpour Ph.D[72] in their study on Ginger state that the it can be useful in the management of polycystic ovarian disease as there was significant increase in level of progesterone in the group treated with ginger extract. Further they state that the ginger in higher dose has similar action like clomiphene citrate. They also suggest that higher dose of ginger can be an alternative for clomiphene citrate which has side effects. Hence Pippalyadi yoga having Ginger can be used to induce ovulation.

NAGAKESHARA

Poonam Arora et.all[73] state that methanolic extract of dried flowers of M. ferrea (100 and 200 mg/kg) showed significant increase in liver SOD and AST, and reduction in catalase, GPX, GR, and ALT activity without any effect on CPK and

creatinine levels in hepatotoxicity induced in S. aureus infected animals. Antioxidant activity in in-vitro and in vivo experiments, M. ferrea expressed antioxidant activity mediated by inhibition of nitric oxide (NO), and lipid peroxidation. In other in vitro antioxidant studies, ethanolic extracts of stem bark of Mesua ferrea Linn. showed significant radical scavenging activity against DPPH (89.70%), ABTS (77.64%) and nitric oxide 89.28%. This present study was aimed at investigation of the effect of D.W extract of Mesua ferrea on gonadotropins showed that Mesua ferrea L. contains phytochemical contents such as glycosides, flavonoids terpenoids and alkaloids. Alkaloids and flavonoids have been shown to reduction serum concentration of LH, estradiol and FSH.

The flower extract of M.ferrea has been shown to possess estrogen and Progesterone like effects which were proposed to be helpful in the treatment of menstrual disorders.[74] The effect of administration of aqueous extract of M.ferra flowers extract at 100, 200 and 300 mg kg- body weight for 21days on the concentration of serum reproductive hormone (Estrogen and Progesterone) was increased in all treated groups when compared with the control, but LH, FSH and prolactin concentrations were decreased after the first, second and third dose when compared with the control. At 21st day low dose (100mg/kg body weight) administration produced high significant effect on the serum hormone The concentration of estrogen and progesterone in the serum was increased by the extract, and the concentrations of gonadotropins and prolactin were decreased by the extract when compared with control. The least dose (100 mg /kg body weight) on the 21st day produced high significant change ($p>0.05$) in the concentration of the hormones. However, by the end of the treatment period, the concentration of estrogen and progesterone had increased by 20%, 24% and 31% respectively in all treated groups when compared with control.

Nagakeshara has the karmas like Uttejaka, Raktasthapaka, Deepaka, Pachaka, Kphagna, Vrushya. Flower extract of M. ferrea has also been shown to possess estrogen and progesterone like effects which were proposed to be helpful in the correction of hormonal imbalance during menstrual disorders.[75]

MARICHA

Maha et al[76] state that noticeable raise was seen in the level of FSH and LH in different doses when compared with normal saline treated mice. There was no significant damage seen in female ovaries tissue. It was also observed that there were increasing number of follicles seen in ovary and significant formation of corpus luteum.

MODE OF ACTION - DRUG PIPPALYADI YOGA.

Pippalyadi yoga is a combination of Trikatu and Nagakeshara with Katu , Tikta and Kashaya rasa and Ushna Veerya . Pippali and Shunti with Madhura Vipaka, Snigdha Guna have shown promising results in the present study which is evident by the increase in estrus phase with signs of folliculogenesis.

Katu rasa is having Deepana , Pachana , Srotoshodhak and also Lekhana properties corrects Agnidushti and digests Aama then clears the Srotas which is obstructed by vitiated Kapha dosha and act as Srotoshodak. This action is responsible for the proper formation of Rasadi dhatus and Upadhatu Artava . So, it corrects Artava Dushti. Sroto Shodhaka Guna of the drugs clears the inflammation in the cells and clears the path way of Beeja Rupi Artava thus initiates the Beejotsarga. Thus, Pipplyadi Yoga is proved to have effect on ovulation by promoting the maturation of the follicles.

PIPPAYADI YOGA WITH ANTI-OXIDANT PROPERTY

According to Agarwal et all[77] Oxidative stress affects physiology of reproductive functions starting from maturation of ovulation fertilization, development of embryo and maintenance and continuation of pregnancy. It is suggested that the decline in fertility as the age advances is due to oxidative stress. The authors also state that the oxidative stress may cause oocyte damage in the follicles. They also harm the oocytes, spermatozoa and also may harm embryo. There also may be corpus luteum regression leading to failure of implantation of embryo due to lack of progesterone.

The drugs in Pippalyadi Yoga have the property of anti-oxidant and support the process of foliculo-genesis and hence prevents the harmful effects of oxidative

stress on oocytes or the embryo.

Neha Choudhary and Vikram Singh[78] quote those pharmacological properties of Pippali also include antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, anti-microbial, anti-platelet, anti-hyperlipidemic, analgesic, anti-depressant, anti-amoebic, anti-obesity, radioprotective, cardioprotective and anti-fungal actions. Network analysis of Pippali shows that 99% of the phytochemicals are linked with more than 10 protein targets suggesting the action of the drug in different levels.

CHAPTER 8

CONTRIBUTION TO SCIENCE AND SOCIETY

In Ayurveda there are elaborated explanation about cause, types and treatment are available. These are well trusted tested and documented. But in this modern scientific era each such treatment need evidence. So, in this regard this experimental study will help the clinicians to support their treatment protocol with positive results towards evidence of ovulation. As infertility is increasing in the society the couple seeking treatment for conception will benefitted by the use of Pippalyadi yoga with Ghritha in case of anovulatory infertility.

CHAPTER 9

CONCLUSION

Infertility is observed in approximately 10-15% of couples of reproductive ages (speroff etall1999). Ovulation disorders account for about 30-40% of female infertility and about 20% of infertility couple(Berna Gunea Suruhan et. All) Anovulation is caused due to the defect in the function of hypothalamus -pituitary- ovarian axis. Artava is considered as ovum and it is formed as a Upadhatu after the proper digestion of Ahara rasa by Prakruta Agni.if Agni is disturbed by Ahita Ahara vihara formation of Rasadi dhatu there by Artava is also affected causing Nastartava which can be considered as anovulation. Different ovulation induction drugs are in clinical use to treat the anovulation. The condition Nastartava also can be managed by Deepana Pachana and Artava Janaka line of management to correct the Samprapthi and to restore fertility.

Pippalyadi yoga is one such formulation mentioned in the management of Vandyatwa with Beeja rupi Artava Dusti in the Bhaishajya Ratnavali Strirogaadhikaara .

This animal study was done to assess the effect of Pippalyadi Yoga (Trikatu and Naagakeshara) on anovulation in Albino rats. The data generated during the study was in accordance with null hypothesis. Anovulation was induced by Norethisterone in this experimental study and treated with Pippalyadi yoga with Ghritha through oral route.

In the present study there was positive changes in estrus phase and also histology of uterus and weight of ovaries. This suggests the drug was useful in the promotion of development of follicles and inducing the ovulation. For the ovulation there should be negative feedback from estrogen which stimulates the synthesis of follicular stimulating hormones needed for the development of the Graffian follicle. In this experimental study there was significant decrease in proestrus phase followed by significant increase in estrus phase suggesting the anti estrogenic action of the drug needed for the negative feedback for the ovulation.

The drugs in Pippalyadi yoga have antioxidant properties which in turn helps in folliculogenesis by reducing the oxidative stress.

The ovulation induction drugs from conventional management have side effects like hepatotoxic and carcinogenesis. The drugs used in this experimental study have hepatoprotective and anti-cancerous properties and hence can be used safely.

AS the rat is a mammal and estrus cycle in rats and menstrual cycle in human are same, the said formulation can be used in humans for the treatment of infertility due to anovulation.

CHAPTER 10

SUMMARY

The thesis entitled "Assessment of Efficacy of Pippalyadi Yoga on Norethisterone Induced Anovulation in Albino Rats" comprises of following chapters.

As the drug Diethylstilbestrol is banned from the market, Norethisterone was taken to induce anovulation.

Chapter 1: Introduction

This chapter gives a general idea about the infertility including incidence, causes of the female infertility, current treatment in practice for anovulation and its side effects, thus enumerating the need for current study.

Chapter 2: Aims and Objectives

Aims to evaluate the effect of Pippalyadi Yoga on Norethisterone induced anovulation in Albino Rats.

Objectives of the study are

1. Phytochemical Analysis of Pippalyadi Yoga
2. Acute toxicity study of Pippalyadi Yoga
3. Efficacy study of Pippalyadi Yoga

Chapter 3: Research question and hypothesis

Research question – Is Pippalyadi Yoga effective in the management of Norethisterone induced anovulation in Albino Rats.

Hypothesis

1. Research hypothesis –Pippalyadi Yoga with Ghritha is effective in Norethisterone induced anovulation in Albino Rats.
2. Null hypothesis – Pippalyadi Yoga with Ghritha is not effective in Norethisterone induced anovulation in Albino Rats.

Chapter 4: Previous works done

In this chapter research works on management of anovulation with different drugs and Pippalyadi Yoga with phytochemical analysis are mentioned.

Chapter 5: Literary Review

Review of literature is subdivided into Ayurveda review, drug review, modern review and explained in detail with respective references. In Ayurveda review references on Vandyatwa with Nidana,Samprpti,Samprpti Ghatakas, Chikitsa Sidhanta and samprapti Bheda are explained. Role of Tridosha in causing Vandyatwa along with role of Satva explained. In Drug review details like habitat, morphology, family ,botanical name, synonyms, Rasapanchaka, Doshaghnata, Rogaghnata of Pippali, Maricha, Shunti and Nagakeshara. Research updates were also mentioned. In modern review anatomy of human ovary and reproductive system of Albino rats along with physiology of ovulation explained. Incidence of female infertility with causes for male and female infertility with treatment of female infertility explained.

Chapter 6- Research Methodology

Methods of Phytochemical analysis and HPTLC of Trikatu and Nagakeshara and Acute toxicity study included. In the methodology of efficacy study animal grouping in 6 groups with 6 albino rats in each group is mentioned. Test drug dosage, preparation, drug administration mentioned. Observation parameters, procedure of vaginal smear taking, blood sampling method and dissection procedure explained. Statistical analysis using oneway Anova followed by Dennett'multiple "t" test was mentioned.

Chapter 7- Analysis and interpretation

In this chapter the interpretation of results of Phytochemical analysis and HPTLC is done.

The results of Acute toxicity study also explained which says no side effects observed with Pippalyadi yoga in Albino rats.

Analysis and interpretation on the results of efficacy study done in detail for all the four phases of estrus cycle. The effect of Pippalyadi Yoga on the serum and ovarian cholesterol, estrogen progesterone, body weight, uterine and ovarian weight is discussed in detail by comparing the test drug with positive control group of Norethisterone. Analysis and interpretation was done on the histopathological changes in the endometrium, ovarian stroma, development of follicles, and vaginal epithelium.

The mode of action of the drugs Pippali, Maricha, Shunti and Nagakeshara is explained in detain with Ayurveda parameters. Research evidences are also mentioned wherever needed.

Chapter 8- Contribution to society and science

this experimental study will help the clinicians to support their treatment protocol with positive results towards evidence of ovulation. As infertility is increasing in the society the couple seeking treatment for conception will benefitted by the use of Pippalyadi yoga with Ghritha in case of anovulatory infertility

Chapter 9-Conclusion

The conclusion of the study is the use of Pippalyadi yoga is beneficial in promoting folliculogenesis in rats. So this combination of drugs can be tried in human as it does not exhibit any side effects.

Chapter 10 - Summary

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PRO FORMA FOR RECORDING SIGNS AND SYMPTOMS DURING GROSS BEHAVIORAL STUDY

Group: 175mg/kg **Dose: 1.58ml**

Animal: Wistar albino rats: **Route: oral** **Drug:** Pippalyadi yoga

SIGNS & SYMPTOMS		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	Active	Active	Active	Active	Active	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion:	Tonic	-	-	-	-	-	-	-	-
	Clonic	-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-		-
	Watery	-	-	-	-	-	-		-
Respiration	Stimulation	-	-	-	-	-	-		-
	Depression	-	-	-	-	-	-		-
	Failure	-	-	-	-	-	-		-
Skin colour	Blanching	-	-	-	-	-	-		-
	Cyanosis	-	-	-	-	-	-		-
	Vasodilatation	-	-	-	-	-	-		-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
mucus membrane		N	N	N	N	N	N	N	N
Piloerection		N	N	N	N	N	N	N	N

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PRO FORMA FOR RECORDING SIGNS AND SYMPTOMS DURING GROSS BEHAVIORAL STUDY

Group: 550mg/kg **Dose: 1.62ml**
Animal: Wistar albino rats: **Route: oral** **Drug:** Pippalyadi yoga

SIGNS & SYMPTOMS		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	Active	Active	Active	Active	Active	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion:	Tonic	-	-	-	-	-	-	-	-
	Clonic	-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-		-
	Watery	-	-	-	-	-	-		-
Respiration	Stimulation	-	-	-	-	-	-		-
	Depression	-	-	-	-	-	-		-
	Failure	-	-	-	-	-	-		-
Skin colour	Blanching	-	-	-	-	-	-		-
	Cyanosis	-	-	-	-	-	-		-
	Vasodilatation	-	-	-	-	-	-		-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
mucus membrane		N	N	N	N	N	N	N	N
Piloerection		N	N	N	N	N	N	N	N

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PRO FORMA FOR RECORDING SIGNS AND SYMPTOMS DURING GROSS BEHAVIORAL STUDY

Group: 2000mg/kg **Dose: 1.56ml**

Animal: Wistar albino rats: **Route: oral** **Drug:** Pippalyadi yoga

SIGNS & SYMPTOMS		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	Active	Active	Active	Active	Active	N	N
Increased motor activity		-	-	+	+	+	+	-	-
Convulsion:	Tonic	-	-	-	-	-	-	-	-
	Clonic	-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-		-
	Watery	-	-	-	-	-	-		-
Respiration	Stimulation	-	-	-	-	-	-		-
	Depression	-	-	-	-	-	-		-
	Failure	-	-	-	-	-	-		-
Skin colour	Blanching	-	-	-	-	-	-		-
	Cyanosis	-	-	-	-	-	-		-
	Vasodilatation	-	-	-	-	-	-		-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
mucus membrane		N	N	N	N	N	N	N	N
Piloerection		N	N	N	N	N	N	N	N

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PRO FORMA FOR RECORDING SIGNS AND SYMPTOMS DURING GROSS BEHAVIORAL STUDY

Group: 2000mg/kg **Dose: 1.60ml**

Animal: Wistar albino rats: **Route: oral** **Drug:** Pippalyadi yoga

SIGNS & SYMPTOMS		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	Active	Active	Active	Active	Active	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion:	Tonic	-	-	-	-	-	-	-	-
	Clonic	-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-		-
	Watery	-	-	-	-	-	-		-
Respiration	Stimulation	-	-	-	-	-	-		-
	Depression	-	-	-	-	-	-		-
	Failure	-	-	-	-	-	-		-
Skin colour	Blanching	-	-	-	-	-	-		-
	Cyanosis	-	-	-	-	-	-		-
	Vasodilatation	-	-	-	-	-	-		-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
mucus membrane		N	N	N	N	N	N	N	N
Piloerection		N	N	N	N	N	N	N	N

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PRO FORMA FOR RECORDING SIGNS AND SYMPTOMS DURING GROSS BEHAVIORAL STUDY

Group: 2000mg/kg Dose: 1.54ml
Animal: Wistar albino rats: Route: oral Drug: Pippalyadi yoga

SIGNS & SYMPTOMS		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	Active	Active	Active	Active	Active	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion:	Tonic	-	-	-	-	-	-	-	-
	Clonic	-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-		-
	Watery	-	-	-	-	-	-		-
Respiration	Stimulation	-	-	-	-	-	-		-
	Depression	-	-	-	-	-	-		-
	Failure	-	-	-	-	-	-		-
Skin colour	Blanching	-	-	-	-	-	-		-
	Cyanosis	-	-	-	-	-	-		-
	Vasodilatation	-	-	-	-	-	-		-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
mucus membrane		N	N	N	N	N	N	N	N
Piloerection		N	N	N	N	+	+	N	N

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Pro forma for assessing Gross behaviour

Drug: Pippalyadi yoga
Group: 175mg/kg Route: Oral
Animal: Wistar albino rats Study: OECD- 425

Time interval	Rat No. 1	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmos	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

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Pro forma for assessing Gross behaviour

Drug: Pippalyadi yoga
Group: 550mg/kg Route: Oral
Animal: Wistar albino rats Study: OECD- 425

Time interval	Rat No. 2	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmos	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rearing
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rearing
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rearing
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rearing
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

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Pro forma for assessing Gross behaviour

Drug: Pippalyadi yoga
Group: 2000mg/kg Route: Oral
Animal: Wistar albino rats Study: OECD- 425

Time interval	Rat No. 3	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmos	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	Rearing
2h			-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	Rearing
3h			-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	Rearing
4h			-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	Rearing
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

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Pro forma for assessing Gross behaviour

Drug: Pippalyadi yoga
Group: 2000mg/kg Route: Oral
Animal: Wistar albino rats Study: OECD- 425

Time interval	Rat No. 4	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmos	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Pharmacology laboratory
SDM Centre for Research in Ayurveda and Allied sciences, Udupi.

Pro forma for assessing Gross behaviour

Drug: Pippalyadi yoga
Group: 2000mg/kg Route: Oral
Animal: Wistar albino rats Study: OECD- 425

Time interval	Rat No. 5	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmos	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Master chart

Pro estrous:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	3	9	6	1	2	3
2	1	8	1	2	3	2
3	2	3	3	1	1	4
4	5	8	4	1	1	2
5	2	2	2	1	2	1
6	3	3	1	3	1	1

Di estrous:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	12	3	4	8	7	3
2	14	6	5	7	5	4
3	8	7	6	4	4	5
4	10	8	6	6	3	3
5	11	3	3	3	5	2
6	14	7	7	3	6	4

Meta estrous:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	2	8	4	2	5	4
2	3	2	3	7	6	3
3	8	3	6	6	7	7
4	5	3	4	6	5	3
5	5	9	3	1	5	1
6	1	2	4	3	6	2

Estrous :

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	4	2	6	8	6	5
2	3	1	8	8	5	6
3	3	2	7	7	5	7
4	1	1	5	5	6	9
5	3	2	4	4	7	8
6	3	1	10	6	4	9

Serum cholesterol:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	49	53	45	53	51	48
2	42	37	54	37	59	56
3	43	50	42	50	64	50
4	52	56	37	56	56	58
5	46	78	34	57	44	68
6	47	89	35	50	68	59

Serum TG:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	28	89	110	125	143	170
2	47	85	115	165	154	100
3	41	89	112	91	109	152
4	55	73	106	129	117	195
5	58	91	134	217	131	213
6	38	87	121	73	124	110

Ovary cholesterol:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	4.6	4.1	4.8	3.9	4.4	4.3
2	3.5	3.5	4.5	5.1	5.1	3.2
3	3.4	3.7	4.4	4.9	5.0	6.4
4	4.6	6.3	4.0	3.8	4.6	4.1
5	3.5	5.8	4.1	3.9	6.1	3.9
6	3.4	6.4	4.9	3.1	5.6	5.2

Ovary TG:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	111	50	56	81	37	70
2	80	45	68	42	71	55
3	98	42	76	40	65	32
4	111	56	64	92	49	89
5	76	43	62	34	44	76
6	98	39	67	61	57	61

Serum Estradiol:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	41	33	32	48	41	41
2	24	34	31	50	39	24
3	45	33	23	42	44	45
4	41	30	30	45	43	41
5	24	34	45	45	37	24
6	45	34	44	50	38	45

Serum Progesterone:

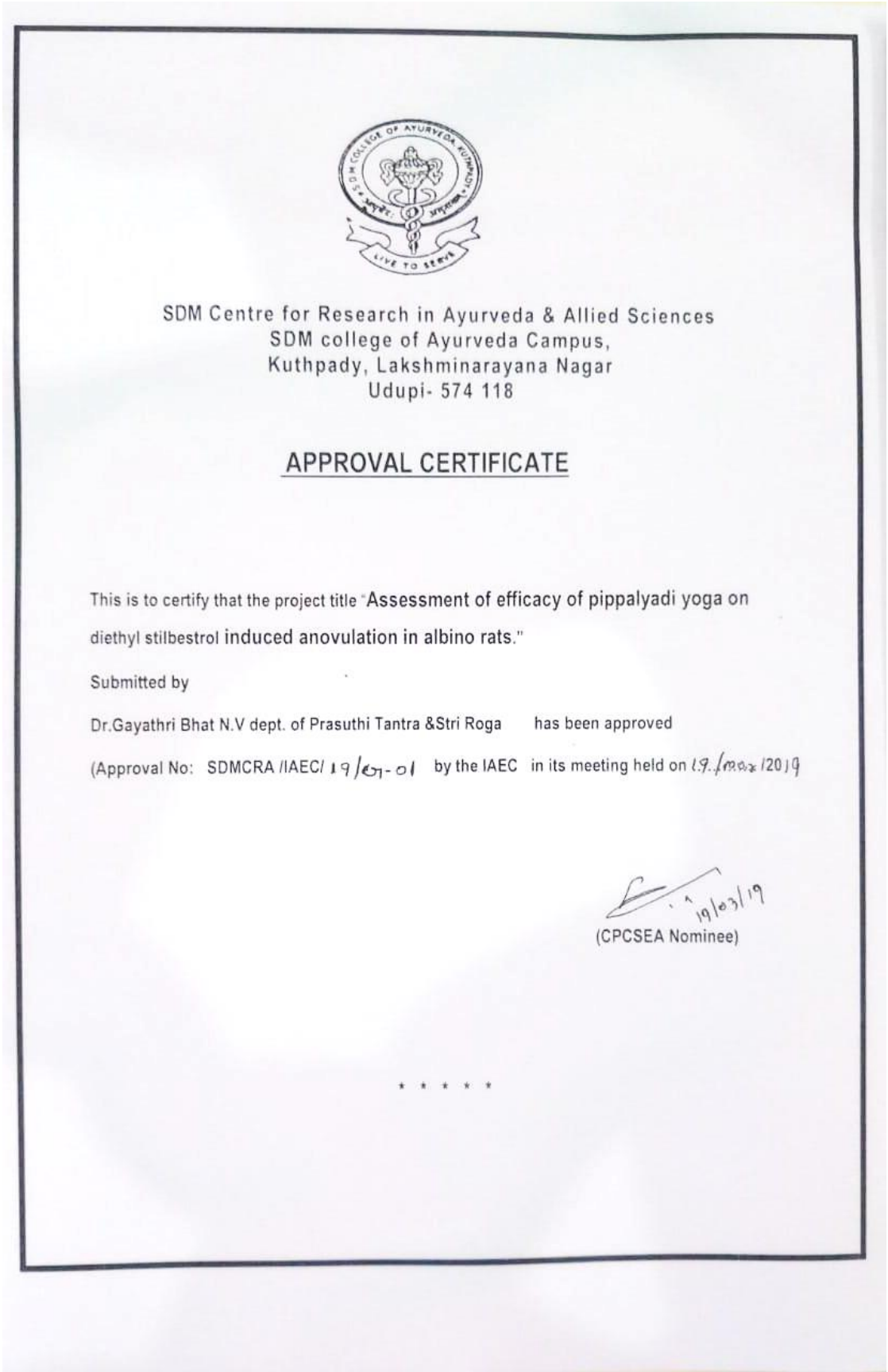
Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	12.4	9.3	10.1	11.2	11.5	8.2
2	15.9	12.9	12.2	10.0	9.8	22.8
3	24.3	11.5	11.4	10.0	8.9	20.0
4	18.4	12.9	10.5	7.8	10.4	10.2
5	15.9	11.5	13.2	7.8	13.2	22.8
6	24.3	9.3	11.9	15.0	12.7	9.2

Wt. of uterus:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	4.9	4.1	4.3	4.6	4.4	3.6
2	3.8	3.5	3.2	3.5	3.8	4.1
3	3.9	3.7	6.4	3.4	3.7	4.8
4	3.1	6.3	4.1	4.6	4.9	3.1
5	4.2	3.5	4.2	3.5	5.1	3.7
6	4.5	2.6	4.4	4.6	4.2	3.2

% change in body wt:

Rat no :	N. Control	PN	G 3	G 4	G 5	G6
1	38.76	32.31	25.75	73.14	17.82	9.05
2	50.71	30.30	49.96	20.57	31.30	4.22
3	48.36	27.43	27.60	20.00	16.67	13.46
4	50.00	13.15	21.08	43.33	14.05	35.55
5	15.00	17.72	9.41	20.86	5.88	17.39
6	36.60	16.33	8.82	4.57	2.94	5.71



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Udupi- 574 118

APPROVAL CERTIFICATE

This is to certify that the project title "Assessment of efficacy of pippalyadi yoga on diethyl stilbestrol induced anovulation in albino rats."

Submitted by

Dr.Gayathri Bhat N.V dept. of Prasuthi Tantra &Stri Roga has been approved

(Approval No: SDMCRA /IAEC/ 19/67-01 by the IAEC in its meeting held on 19/mar/2019

19/03/19
(CPCSEA Nominee)

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