INTRA SPERMIC CALCIUM STORE IN REVERSIBLE STATE OF CALCIUM RIGOR IS RESPONSIBLE FOR SPERM MOTILITY AND POLYSPERMY PREVENTION – A NEW PERSPECTIVE

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Abstracts: Non-pulsatile GnRH during spermatogenesis is responsible for production of less Estrogen [OE] and Progesterone[PR] with more Androgens in male. With relative lack of PR [respiratory alkalosis] induced Ca^{2+} lowering mechanism, Ca^{2+} bioavailability is enhanced in male. Ca^{2+} triggered centriolar spindle contraction favouring mitotic and meiotic divisions of spermatocytes produce millions of haploid spermatids daily and stockpile them in reversible Ca^{2+} rigor state in anaerobic acidic environment of male genital tract. Thus intraspermic Ca^{2+} store is formed. On ejaculation aerobic environment favours spermic mitochondrial ATP production causing Ca^{2+} detachment from its' binding site, maintains continuous supply of active IntraSpermic Ca^{2+}. Resultant continuous Spermic Ca^{2+} efflux triggers semen coagulation, coagulum liquefaction by acrosomal proteolytic enzymes action and restores sperm motility with cyclical binding and releasing of Ca^{2+} from flagellar contractile proteins. Motile sperm penetrate corona radiata of secondary oocyte by proteolytic acrosomal enzyme action and bind to species specific Zona Pellucida [ZP] receptor. On entry, sperm loses membrane releasing active Ca^{2+} intra-oocytically. Resultant Ca^{2+} wave/spark triggers centriolar contraction forming second polar body cause progress in arrested 2^{nd} meiosis of secondary oocyte. Ca^{2+} wave/spark is responsible for fast electrical block and triggers slow block by Ca^{2+} salts deposition at oolemma, prevent further sperm entry.

Key Words: Binding Sites, Biological Availability, Haploidy, Male, Polar Bodies, Sperm Motility.

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Introduction:

A Oocyte (or ovum/egg) activation is a series of processes that occur in the oocyte during fertilization. Sperm entry causes calcium release into the oocyte. The cause of calcium release remains to be established definitively[1 - 8]. **A**ctivation of the ovum includes the following events:

- Cortical reaction to block against other sperm cells
- Activation of egg metabolism
- Reactivation of meiosis
- DNA synthesis

Polyspermy is the condition when multiple sperm fuse with a single egg. This results in duplications of genetic material. Many studies in animals observed the block to polyspermy due to two mechanisms: the fast block [electrical block i.e. from -70mv to +20mv] and the slow block i.e. calcium wave triggered cortical reaction converting vitelline membrane to impermeable fertilization membrane to other sperms by inactivating ZP receptors[1 - 8].

Reactivation of meiosis -

The secondary oocyte remains suspended in metaphase of the second meiotic division. The calcium wave reactivates the meiotic cycle producing and extruding the second polar body[1 -12].

Artificial oocyte activation

Oocyte activation may be artificially facilitated by calcium ionophores, as observed in some cases of failed intracytoplasmic sperm injection [ICSI] cycles[7].Another method is by using the drug Roscovitine, this reduces the activity of M-phase promoting factor activity in mice[8].

Material and Methods:

This review was prepared based on the papers published in PubMed, Google Scholar, indexed

journals and standard anatomy, embryology and physiology textbooks.

The new perspective -

It concentrates on "sperm as the source of Ca^{2+} which triggers all the activity associated with sperm motility and fertilization". The perspective supports all the universally accepted observations in animal and human studies and tries to explain "The mechanism of Ca^{2+} release" with the following revision of facts related to Ca^{2+}[9 - 11].

1] Ca^{2+} is present in both Intra Cellular fluid [ICF] and Extra Cellular fluid [ECF] compartments.

2] Normal ICF concentration is 12000 times less than ECF. So the normal concentration gradient and electrochemical gradient favours Ca^{2+} influx.

3] Calcium is present in two forms i.e.

- ✓ a] 'Ionic form **[biological active form]'** and
- ✓ b] 'Bound form bound to organic and inorganic anions **[store form]**'.

Both forms are interconvertible, depending on pH of the environment. i.e. the H^+ of acidic environment competes with calcium in bound form releasing Ca^{2+} and viceversa in alkaline environment which favours conversion of Ca^{2+} to bound form.

Till today confusion persists to explain the mechanism of motility with calcium influx theory, with acidity decreasing and alkalinity favouring motility within range of 5.2 to 8.2 though the interrelation is well established in all the studies with many cross references supporting the observations[12 -24].

- ✓ Present perspective **explains the sperm motility mechanism as favoured by calcium efflux** with spermic calcium store.

4] The new perspective concept is based on universally accepted observations as follows

A. New perspective concept related to oocyte–

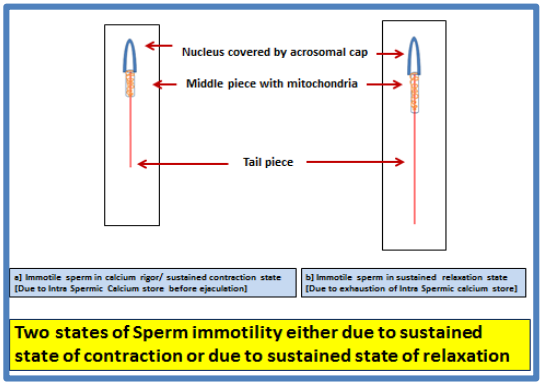
- ✓ **'Less ICF Ca^{2+} content of oocyte' –** due to Ca^{2+} lowering mechanism of PR.

B. New perspective concept related to sperm –

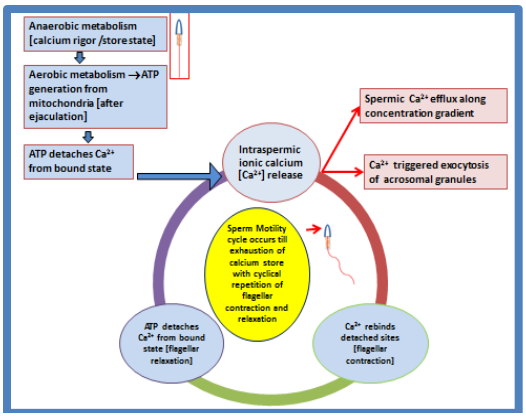
- ✓ **Sperm is source of calcium store -** due to reduced PR with relatively supressed Ca^{2+} lowering mechanism of PR.

C. **New perspective concept of spermic Ca^{2+} efflux mechanism related to sperm motility and polyspermy prevention.**

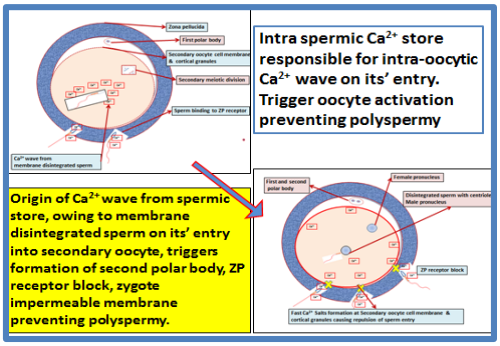
- Maintainance of Active Intra spermic Ca^{2+} with the help of mitochondrial ATP helps in sperm motility by cyclical detachment and attachment of Ca^{2+} to spermic contractile flagellar proteinsand on entry into secondary oocyte produces calcium wave/ spark resulting in **fast electrical block and slow block byformation of calcium salts at oolemma membrane**



- Fig no. 1- **Two states of Sperm immotility either due to sustained state of contraction or due to sustained state of relaxation**. a] Immotile sperm in calcium rigor/ sustained contraction state[Due to Intra Spermic Calcium store before ejaculation]. b] Immotile sperm in sustained relaxation state [as a result of exhaustion of Intra Spermic calcium store by continuous calcium efflux after ejaculation and the rate of efflux decides the force and duration of motility].



- Fig no.2 - **ATP generation from mitochondria [after ejaculation] due to Aerobic metabolism results in continuous Intraspermic ionic calcium [Ca^{2+}] release which restores sperm motility with cyclical repetition of unbinding and binding of calcium and responsible for acrosomal granules release necessary for the semen coagulation, liquefaction of coagulum , penetration of corona radiata and calcium wave of fertilization.**



- Fig no.3- **Origin of Ca^{2+} wave from spermic store, owing to membrane disintegrated sperm on its' entry into secondary oocyte, triggers formation of second polar body, ZP receptor block, zygote impermeable membrane preventing polyspermy.**

Discussion: and evaluation–

Explanation of new perspective -

- **A] New perspective concept related to oocyte– 'Less ICF Ca^{2+} content of oocyte'. –**
  - **Evaluational Proof -**
    - ✓ i] Primary oocyte arrest of first meiotic division in prophase [Diplotene stage]
    - ✓ Ii] secondary oocyte arrest of second meiotic division in metaphase.

i] Accepted observations–[9 - 15]

- During oogenesis there is arrest of first meiotic division in prophase [Diplotene stage], even before birth.
- ✓ During ovulation there is activation of first meiotic division by formation and extrusion of first polar body and
- ✓ Further activation gets arrested during second meiotic division in metaphase.
- ✓ **New perspective Explanationand evaluation related to primary oocyte–**
  - During ovulation there is transient ischaemic condition of dominant follicle as a result of explosive proliferation under preovulatory gonadotropins' surge which induces, intra- oocytic acidic environment. The resultant Ca^{2+} release from intracellular store of primary oocyte triggers formation of first polar body [29 - 32].
  - The further arrest of second meiotic division of secondary oocyte in metaphase is the proof that there is minimal intracellular Ca^{2+} store of oocyte and it gets exhausted during ovulation.

ii] Accepted observations –

- ✓ During fertilization [i.e. entry of sperm into secondary oocyte] calcium wave is generated[1 -15].
  - at the site of sperm entry in secondary oocyte or

- at the site of artificial introduction of sperm extract.

✓ The calcium wave triggers further activation of oocyte by forming and extruding second polar body.

❖ **New perspective Explanationand evaluation related to secondary oocyte–**

- Above observations prove that

✓ ICF Ca^{2+} is necessary for completion of secondary meiosis which is not available because

✓ There is minimal ICF Ca^{2+} store of oocyte which gets exhausted at ovulation.

- As the intraoocytic sperm entry / sperm extract is essential , it can be concluded that **'sperm is the only source of calcium responsible for calcium wave of fertilization'.**

B[New perspective concept related to sperm

- **Sperm is source of calcium store →**

✓ Proof –

i. Cyclic spermic effux of Ca^{2+} is responsible for motility regain of stockpiled sperms after ejaculation and

ii. Calcium wave of fertilization i.e. after sperm entry into secondary oocyte

i] Accepted observations – [9 – 15, 33-36]

✓ **ICF Ca^{2+}** is necessary for both mitotic and meiotic divisions

✓ On meiotic division one diploid primary oocyte forms one haploid mature ovum and three haploid polar bodies where as one diploid primary spermatocyte produces four haploid spermatids.

- Meiotic division of oocyte gets arrested two times. First time at birth and second time after ovulation.**Only after sperm entry there is progression of second meiotic division which was arrested at metaphase.**
- **Calcium wave of fertilization** is responsible for the secondary oocyte activation.

Explanation for "How and why spermatocytes have more calcium bioavailability to facilitate both mitotic and meiotic divisions and for spermic store?"

❖ **Explanation for** sufficient calcium bioavailability to spermatocytes –

✓ Production of oocyte stops at birth whereas sperm production continues in male from puberty till late age and millions of sperms are produced daily. And it proves the fact that there is sufficient calcium bioavailability to spermatocytes in males

✓ During reproductive phase, under the influence of Gonadotropin releasing hormone [GnRH] there is synthesis of sex hormones from gonads i.e. cholesterol gets converted to three groups of hormones like→ progesterone [PR] → Androgen →Oestrogen [OE].

a. Physiologically, the target biochemical substance will have a negative feedback regulation on its' stimulating factor.

b. Biochemically, wheneverthere is block in biosynthesis of target biochemical substance [i.e. OE] there is accumulation of its' intermediary products [i.e PR and androgens].

❖ In females, the negative feedback effect of oestrogen on GnRH creates its' pulsatile nature producing a lot of PR as intermediary product.

❖ Whereas in males as OE is not produced in regular sufficient amount

to cause its' negative feedback on GnRH[29-32].

- ✓ Probably because of on continued repetitive stimuli by the GnRH, there is a relative fatigue state of sex steroid synthesizing cells resulting in accumulation [production] of Androgens as intermediary product.

- In females PR is always more than in males and PR Ca^{2+} lowering action is responsible for the decreased bioavailability of Ca^{2+} in females and increased bioavailability of Ca^{2+} in males. [29 - 32].
  - ✓ Mechanism of PR induced Ca^{2+} lowering action - PR by its' respiratory centre stimulatory effect causes respiratory alkalosis leading to decreased plasma Ca^{2+}.
- ✓ The **better bio- availability of Ca^{2+} in males** might be responsible for triggering the mitotic and meiotic division of spermatocytes to such a great extent that, from one spermatogonia 512 spermatids are produced and millions of sperms are produced in one day

Explanation for the mechanism of motility -

Accepted observation – The millions of haploid motile sperms are formed everyday in seminiferous tubules of male genital tract gets stockpiled [stored] for days and sometimes even for months in epididymis and vasdeferens. They are stored in immotile state in anaerobic metabolic environment with minimal amount of surrounding fluid[13].On ejaculation, motility is regained.

❖ **The new perspective tries to explain sperm motility with the concept of sperm calcium store. And it may be noted that the Ca^{2+} efflux not the influx can only explain the mechanism.**

i. Sperms are reversibly immotile in two conditions i.e. either in sustained state of contraction or in sustained state of relaxation [fig no. 1]

ii. Sperms have flagellar contractile portions in body and tail parts which are modified Ca^{2+} sensitive microfilaments derived from spindle parts of centrioles.

iii. Contraction [i.e. shortening]occurs on Ca^{2+} binding to contractile unit and relaxation [i.e. elongation] occurs by detaching Ca^{2+} from its' bound site.

iv. Both contraction and relaxation require energy[ATP] and is provided by the rich mitochondria present in body of sperm in aerobic environment.

a) **Explanation** for Immotility due to sustained state of contraction is reversible due to reversible calcium rigor

- ✓ After spermatogenesis, sperms are stockpiled in male genital tract in anaerobic acidic conditions which favour intraspermic Ca^{2+} release, favouring binding of Ca^{2+} to contractile units inturn causing reversible calcium rigor state.

  Note – In cryptorchidism and males working in hot environment [at genital organs level] there is infertility. The reason for infertility may be due to irreversible heat rigor occuring at 40^0C [**i.e. irreversible sperm immotility**]. This universal observation proves the presence of sensitive protein at sperm flagella.

b) **Explanation for** Immotility due to sustained state of relaxation is due to complete removal or absence of intraspermic **[ICF]**Ca^{2+} occurs in following well accepted observations.

- ✓ With complete absence / reduced Ca^{2+} in extraspermic fluid **[ECF]**, there is greater gradient for **Ca^{2+}** with enhanced spermic Ca^{2+} efflux triggering faster and complete exhaustion of intraspermic Ca^{2+} .

- **Conlusive explanation for** the duration and strength of motility relation to calcium gradient.
  - ✓ Thus the duration of active sperm motility varies inversely with gradient and

- ✓ The active sperm motility strength varies directly with gradient.

❖ **Following are the universally well accepted observations with the new perceptive conclusions with explanations–**[16 -28]

- 1a] When Extra spermic fluid [**ESF]** $Ca^{2+}$is removed by chelation, motile sperms get converted to immotile state.
- 1b] On readdition of Ca^{2+} to **ESF,** sperm motility was regained.
  - Conlusion –
  - 1a] The observation proves that due to exaggerated Ca^{2+}efflux along higher concentration gradient, the intra spermic [**ICF]** Ca^{2+} gets reduced to such low level that sperm has to relax and remain in state of relaxation [reversible immotility].
  - 1b] And reversal of immotility to motile state with addition of Ca^{2+} to extra spermic environment [ESF], proves the fact of restoration of ICF Ca^{2+} by Ca^{2+} influx along inward directed concentration gradient and further motility is maintained by recycling of Ca^{2+} from attachment and detachment of spermic contractile binding sites.
- 1c]Many studies observed that addition of Ca^{2+} channel blocker along with chelation maintained the motility,
  - ❖ Conclusion –
    - 1c] The observation proves that Ca^{2+} channel blocker blocks the spermic Ca^{2+} efflux [not influx] thus maintains sufficient intraspermic Ca^{2+} necessary for the motility. [fig -2]
- 2] Many studies observed gradually better Sperm motility with enhanced alkalinity of Extra Spermic environment [ECF] from pH 5.2 to 8.2, though actual optimal pH varied with species and for humans it is 7.2 -7.6. The mechanism is thought to be due to alkaline sensitive Catsper family channel.
  - Conclusion – As the Extra Spermic Ca^{2+} level decreases with **Extra spermic environmental [ECF]** alkalinity, there is exaggerated spermic Ca^{2+} efflux along intensified concentration gradient. This enhances the number of active intraspermic Ca^{2+} binding sites available for next successive contraction, resulting in better motility. And this continues till the spermic Ca^{2+} store gets exhausted by its' cyclical repetition.

New perspective of sperm motility mechanism – [fig no 2]

The following accepted observations are summarized .

- ✓ After spermatogenesis motile sperms are stockpiled in male genital tract in immotile state.
- ✓ After ejaculation, there is coagulation followed by liquefaction of semen with regain of motility in 80% of sperms.
- ✓ Motility is favoured in comparative alkaline Extra Spermic environment.
- ✓ Further motility is favoured in female genital tract by alkaline utero-cervical secretions and uterine suction pump.
- ✓ Out of 300 -500 millions of sperms ejaculated, only 300-500 sperms can go near the secondary oocyte.

Explanation –

As explained above, the Calcium bioavailability in men

- ➢ Helps in daily spermatogenesis of millions of motile sperms.
- ➢ And they are stockpiled in male genital tract in reversible immotile state due to calcium rigor.
- ➢ Thus sperm becomes the store house of Intra Spermic Ca^{2+} which is necessary for motility.

On ejaculation, aerobic metabolism is established and the rich spermic mitochondria produce ATP. ATP maintains uninterrupted generation of active Intra Spermic Ca^{2+} by detaching Ca^{2+} from sperm contractile portion. Thus on ejaculation, the active Intra Spermic Ca^{2+} is responsible for the universally observed three functions like instant semen coagulation followed by liquefaction within 15minutes and sperm motility.

Summary of Mechanism of reversal of motility –

Motility is due to cyclical repetition of contraction and relaxation of contractile portion of sperm i.e. flagellum. On aerobic spermic metabolism, rich mitochondria present in sperms provide the necessary energy i.e. ATP.

Aerobic spermic metabolism → ATP generation → maintain ↑Intra Spermic [ICF] Ca^{2+} by detaching Ca^{2+} from its' binding site. Three functions are observed as the outcome of ↑ Intra Spermic [ICF] Ca^{2+} → i.e

i] Part of ↑ Intra Spermic [ICF] Ca^{2+} → triggers release of acrosomal granules; responsible for liquefaction of semen coagulum [within 15 min.] and penetration of corona radiata of secondary oocyte.

ii] Part of↑ Intra Spermic [ICF] Ca^{2+} → effluxes out; responsible for instant semen coagulation after ejaculation

iii] Part of ↑ Intra Spermic [ICF] Ca^{2+} → used in rebinding of $Ca^{2+}$to the open sites of contractile unit resulting in contraction.

- ❖ All-over again ATP helps to detach Ca^{2+} [relaxation] → ↑ Intra Spermic [ICF] Ca^{2+} → rebinding of $Ca^{2+}$to the open sites [contraction] →
- ❖ Produces cyclical repetition of contraction and relaxation i.e. sperm motility. →
  a) Preservation of motility is as long as Intra Spermic [ICF] Ca^{2+} store is present.
  b) Duration of motility is limited by the cyclical Ca^{2+} efflux induced exhaustion of ISF Ca^{2+}

The above details explain calcium store of motile sperm which leads to following events.

- ✓ The motile sperm by continued spermic calcium efflux is able to penetrate corona radiata of the secondary oocyte by the proteolytic action of acrosomal enzymes and bind to species specific Zona Pellucida [ZP] receptor.
- ✓ There is calcium wave originating in the secondary oocyte at the site of sperm entry and spreads to its' periphery [01-15] .

- ❖ **New perspective explains mechanism of prevention of polyspermy as follows.**
  - ✓ 1] Fast electrical block - occurs due to releaseof calcium [Ca^{2+}] spark/ wave which instantly converts the membrane potential from -70mv to +20 mv.
  - ✓ 2] Slow block - occurs due to calcium [Ca^{2+}] spark/wave spreading to the periphery of secondary oocyte and triggering
    - The progress of second meiotic division of secondary oocyte by favouring contraction of spindle of centrioles to form and extrude the second polar body.
    - Decking of both cortical granules of oocyte and spermic acrosomal granules together, which take part in converting the permeable oolemma membrane to impermeable one as follows.

i. The fertilized oocyte membrane undergoes chemical changes under the influence of proteolytic enzymes of cortical [oocyte origin] and acrosomal granules of spermic origin

ii. There is configurational change of ZP receptor along with change in egg membrane after combining with Ca^{2+} to form salts. These Ca^{2+} salts are similar to the egg shell membrane of other viviparous animals and make it impermeable.

iii. Calcium salts of impermeable membrane of oolemma forms the source of Ca^{2+} which are released intermittently as calcium oscillations.

Proof –

- the cleavage division of fertilized ovum upto marula stage i.e for 5 days and the outer wall of egg breaks at 60-100 cell stage occurs with gradual dissolution of membrane.
  - ✓ The observed oscillations of Ca^{2+} might be derived from the dissolution of calcium salts of the zygote membrane. It must be due to anaerobic metabolism of enclosed space of fertilized egg creating acidic environment and triggering the cleavage mitotic division of zygote.

Conclusion:

Comparative non pulsatile nature of GnRH stimulation during spermatogenesis is responsible for less production of OE and PR and more

accumulation of Androgens in male. Relative absence of PR induced active Ca^{2+} lowering mechanism, results in enhanced bioavailability of Ca^{2+} in male. It favours the mitotic and meiotic division of spermatocytes for daily production of millions of haploid spermatids and their storage in reversible immotile state due to Ca^{2+} rigor in the anaerobic environment of male genital tract. Thus sperm acts as Ca^{2+} store and on ejaculation, the aerobic environment with production of ATP from spermic mitochondria detach Ca^{2+} from its' binding site releasing active Intra Spermic [ICF] Ca^{2+}. This favours spermic Ca^{2+} efflux resulting in semen coagulation, liquefaction of coagulum by release of acrosomal proteolytic enzymes and with cyclical binding and releasing of Ca^{2+} from flagellar contractile proteins result in sperm motility. The motile sperm is able to penetrate by its' proteolytic action on corona radiata cell layer of the secondary oocyte binding to species specific Zona Pellucida [ZP] receptor. On sperm entry spermic Ca^{2+} is released into secondary oocyte cytoplasma , producing Ca^{2+} wave which triggers fast electrical block due to depolarisation and also slow block due to Ca^{2+} salts deposition at oolemma membrane, making it impermeable, prevents polyspermy and results in progression of 2^{nd} meiotic division of secondary oocyte with formation of second polar body.

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