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Reflections on the state of the art of human reproduction

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The field of human reproduction is a rapidly changing one and as more areas are explored new challenges come into focus. (A detailed reflection of these changes constantly attract a hole textbook publication). The aim of this paper is to consider some of the major advancements in both male and female reproduction with particular emphasis on those that have immediate relevance to the clinical treatment of infertility. The most recent statistics indicate that 300 million couples have no access to family planning and there are some 60-80 million infertile couples world-wide in a world population of 5.5 billion[1].

The female reproductive system

Ovulation

Perhaps the most central focus of the female reproductive system is ovulation. For more than half a century ovulation has been known to be dependent on a complex neuroendocrine mechanism between the hypothalamus and the pituitary gland. It is now well known that ovulation occurs as a result of the action of hypothalamic LH-RH (luteinizing hormone releasing hormone) on the pituitary gland to cause pituitary LH (luteinizing hormone) and FSH (follicle stimulating hormone) release which in turn now act on the ovary to cause ovulation[2]. Equally important was the observation that exogenous FSH can cause endogenous FSH release with multiple follicular development and partial ovulation[3]. More recently it became clear that the action of LH and FSH on the ovary to cause ovulation depends on some intraovarian neurohumoral activities. Together it can be said that the ovarian paracrine/autocrine control mechanism include activin, folliculostatin, insulin-like growth factor (IGF), interleukin-1 and epidermal growth factor (EGF) to mention a few[4,5]. On the ovary the action seems to be on the granulosa cell rather than the theca cells. In addition at the brain level, opoid peptides of the hypothalamus affect LHRH release by their sensitivity to exogenous (light, stress) or endogenous (cytokines, steroid hormones, B-endorphins) stimuli[4]. Figure 1 shows a diagramatic representation of the control mechanisms.

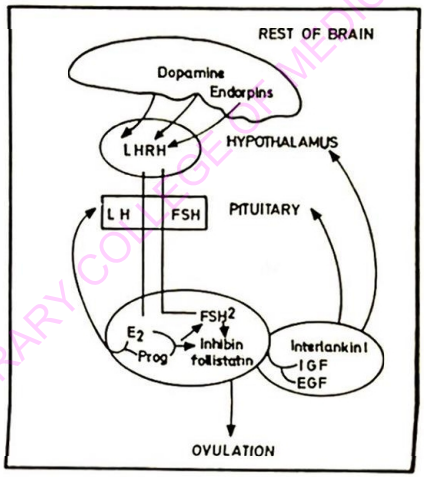

Fig 1: Diagramatic representation of the control mechanism of LHRH release and ovulation

Superovulation or hyperstimulation

The understanding of the above ovulatory mechanism has enabled scientists to cause multiple ovulation in animals and humans. Successful superovulation in animals[6] has enabled its implementation in humans. The need for superovulation or hyperstimulation in humans became more important with the advent of in-vitro fertilization and embryo transfer (IVF-ET) as a treatment of infertility, and in anovulatory patients. The evolution of the hyperstimulation protocols over the last five years has been dramatic. Some of the many protocols used are shown in Table 1. In general the most recent involves the use of LHRH analogues (e.g. Buserelin), pure FSH (metrodin) or Human Menopausal Gonadotrophin (HMG) Pergonal,

* Correspondence as above

Human Chorionic Gonadotrophin (HCG) and prevention of ovarian hyperstimulation[7]. We have found the use of the short LHRH analogue prior to the use of pure FSH or HMG to be very good in producing good oocytes[8]. There is also recently the use of recombinant FSH[9].

Table 1: Superovulation protocols

A. Clomid 50 - 200mg daily for 5 days
   plus HCG 5000 I.U. on day 12.
B. Clomid 50 - 200mg daily for 5 days (D2 - 6)
   plus Pergonal or Metrodin injection (75 - 225 I.U.) between (D3 - II)
   plus HCG 5000 - 10,000 I.U. on day 12.
C. LHRH agonist e.g. Buserelin short or long protocol
   plus HMG or FSH and HCG.
D. Growth hormone, LHRH agonist, + HMG or FSH and HCG.

Further use for LHRH analogues

It is necessary at this point to give some time to the evolution of LHRH in Medicine. Numerous clinical uses in female and male reproductive systems have now been established for analogues of the hypothalamic LH- and FSH- releasing hormones (LHRH or GNRH and FSH-RH). The advances in this field were made possible by the isolation, determination of structures and synthesis of LHRH by Schally *et. al.* in 1973[10]. Schally got a Nobel Prize for this in 1977. The process of administering LHRH agonist termed pituitary down-regulation or medical hypophysectomy have found place in the treatment of endometriosis, uterine leiomyoma (fibroid), polycystic ovarian disease (PCOD) syndrome, hyperstimulation in IVF-ET or GIFT or other forms of assisted conceptions (HAC). Some of the agonists are equally useful in the treatment of breast cancers that are oestrogen sensitive, prostatic cancer, ovarian cancer and other endocrine-dependent or hormone-sensitive tumours[11].

Male reproductive system

While the process of spermatogenesis is equally dependent on the control of hypothalamic LHRH and pituitary LH and FSH release, a number of local factors at the intra-testicular level have become the subject of several investigations. The "sperm factor" constitutes about 40% of infertility and this is due to abnormalities of the male reproductive system. This could occur as a result of one or a combination of the following factors:

(a) Problems associated with the spermatogenic process.
(b) Congenital or ductal pathology.
(c) Semen factors (oligo/azo/astheno/terato-spermia) meaning abnormal count, motility and morphology.
(d) Coital problems.

Problems associated with the spermatogenic process vary from inadequate hormone production as in hypogonadism, infection and drug toxicity. Chloroquine has been reported to affect male reproductive capacity[12]. In a preliminary study salicylate was found to decrease sperm motility in-vitro and in-vivo[13]. In the chloroquine study, we employed a battery of tests for efficient evaluation of the effect of chloroquine on testicular morphology using efficient and reliable stereological methods. The results suggest that chloroquine exerts an inhibitory effect on spermatogenesis, as evident by decrease in total tubular surface area and volume-weighed mean volume (Table 2) as well as impair the function of the Leydig cells which lead to a decrease in weight of androgen dependent tissues[12]. Figure 2 shows the morphological alterations in chloroquine treated rats. Similar observations were made suggesting that chloroquine suppresses ovulation[14]. It was also observed that Aspirin equally suppressed ovulation[15] probably by inhibiting the whole process of follicular development and rupture (Fig. 3). Causes of male infertility from ductal pathology include undescended testis[16], varicocoele, poorly treated venereal diseases and delayed treatment of torsion of the testis and sickle cell disease[17].

Improvement of sperm profile

The treatment of male infertility has been mainly through elimination of causes in treatable conditions, prevention by avoiding such drugs as already identified, and the use of loose underwears to encourage a lower temperature favourable for the enzymatic activities needed for spermatogenesis. Presently, pilot studies with testosterone, clomid, LHRH analogue, gonadotrophin, (pure FSH versus HMG), human gonadotrophin to treat male infertility in hypothalamic-pituitary-testicular axis disorders are in progress in our laboratory and other parts of the world. Although preliminary results obtained by us are encouraging, the overall results of the effectiveness of the novel therapy are still being

collected and evaluated. When the male infertility is due to primary testicular failure, this can be alleviated using donor inseminations.

Table 2: Effect of chloroquine on total number of spermatocytes, volume-weighed mean seminiferous tubular volume (Vv), surface area (S) and weights of androgen dependent organ

Parameter	Control	Chloroquine Treated 10mg	40mg
Total Spermatocyte Count ($x10^6$)	105.0 ± 26.5[a]	1352.5 ± 391.0[b]	2927.5 ± 317.0[c]
Vv (cm^3)	266.35	95.77	95.77
S (mm)	106.0 ± 12	83.0 ± 18[d]	73.0 ± 6[c]
Testis (mg)	2.4 ± 0.16	2.6 ± 0.53	2.5 ± 0.46
Prostate (mg)	0.7 ± 0.11	0.49 ± 0.07[e]	0.48 ± 0.12[e]
Combined Weight of Seminal Vesicle and Coagulating Gland (mg)	1.22 ± 0.26	1.04 ± 0.29	1.43 ± 0.16

a = mean ± S.D.
b = $P < 0.005$
c = $P < 0.0001$
d = $P < 0.01$
e = $P < 0.05$

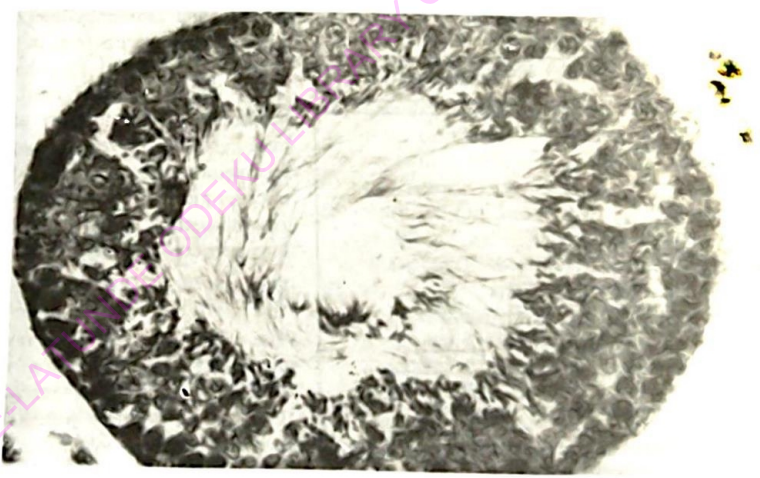

Fig. 2 (a) Testicular morphology of chloroquine treated rat (x 400 H & E stained). The seminiferous tubules show mid-zone degeneration.

Further techniques in sperm technology

The process of artificial insemination has been highly modified and improved especially in the treatment of oligospermia, oligo-asthenospermia or oligo-asthenoteratospermia. The first improvement was sperm washing and capacitation (swim-up) technique[18] which enables normal sperm to be separated from the others. The good separated spermatozoa are now inseminated as intrauterine (IUI) or intratubal (ITI) inseminations. Figure 3 shows sperm profile pre- and post-capacitation. The prognosis has been very good. In our laboratory the pregnancy rate is up to 60% in 3 cycles. This is quite similar to report by a multicenter study[19].

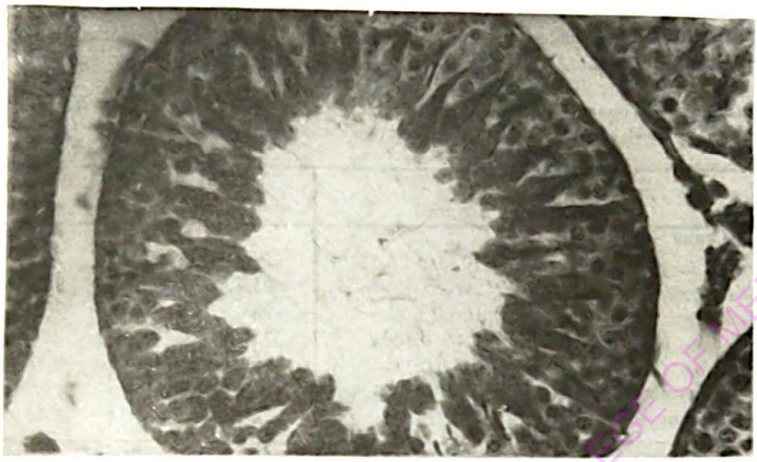

Fig. 2(b) Testicular tissue morphology of control rat (x 400 H & E stained). Seminiferous tubular epithelial cell height, size and lumen appears normal. The lumen is filled with spermatozoa.

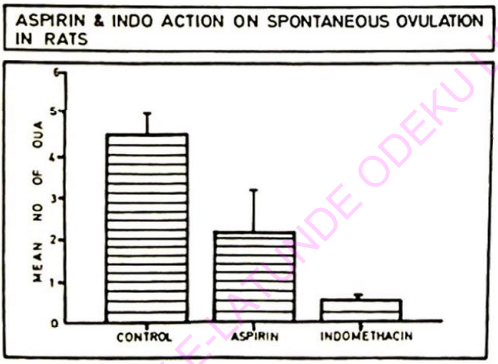

Fig. 3: Aspirin and indomethacin action on spontaneous ovulation in rats.

Table 3: The effect of capacitation on human sperm count and motility

Parameter	Before Capacitation	After Capacitation
Sperm count ($x10^6$/ml)	66.67 ± 0.00[a]	52.00 ± 2.78
Motility (%)	48.00	58.00

a = mean - S.D.

Apart from this there is the multiple ejaculate collection and concentration of sperm especially with cryopreservation[20]. Equally helpful is the use of xanthines that enhance motility like pentoxifylline, caffeine and aminophylline[21]. These have been reported with success (e.g. in oligospermic patients). Other methods used to enhance sperm fertilizing ability include the glasswool filtration, Percoll for maximizing count, motility and normal forms, and mini-Percoll for severe oligospermia. They all show an enhanced pregnancy rate. Finally, the process of microsurgical epididymal sperm aspiration, partial zona dissection, and sperm micro-injection (SUZI) have proved very useful[7].

The ovum donation

The whole story of human reproduction will not be complete without mention of the success now realised from therapies with oocyte donation. It has been found useful in patients with ovarian failure, resistant ovarian syndrome, genetic defects and multiple ovarian cyst. The donor age is usually under 35 years, recipient age has varied from 24-29 years. The pregnancy rate in most centers range from 25% - 33%, and it is not affected by age[22,23]. More recently, growth hormone is now being used in inducing ovulation especially in premenopausal women and in difficult anovulatory patients. GH is very expensive and therefore limited in use. However, it seems very effective in causing ovulation. We have encouraging result with this procedure in Lagos[8].

Conclusion

It is very clear that the state of human reproduction is a dynamic process. The knowledge in this field increases daily and no nation or group of scientists should attempt to be left behind. There is no doubt that increased knowledge this field would ensure proper demographic control, good health and well being. It is obvious that very soon it will be possible for any man or woman to have his/her own child. Equally soon less complicated, easy and acceptable means of birth control will emerge. The race continues in this area of human reproduction. A lot still needs to be learned. One can only request for a great deal of ethical consideration and restraint on the part of medical scientists as the research on embryo manipulation and engineering continues.

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