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RESEARCH ARTICLE

A CRITICAL REVIEW OF GONADOTROPIC ACTIVITY OF PREGNANT MARE SERUM GONADOTROPIN

[1]Nikki Kumari, [1]Taruna Arora, [1]Sharmila Basu Modakand [*2]Kambadur Muralidhar

[1]Department of Zoology, University of Delhi, Delhi-110007, India
[2]Faculty of Life Sciences andBiotechnology, South Asian University, New Delhi-110021, India

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ABSTRACT

The equine placental hormone (eCG), otherwise also referred to as Pregnant Mare Serum Gonadotropin (PMSG, the serum component) is a glycoprotein hormone with the highest content of carbohydrate among the pituitary and placental gonadotropins. Studies have revealed that PMSG acts like Luteinizing Hormone (LH) in mare/horse but exhibits both LH and Follitropin (FSH) like activities in heterologous animal species. PMSG is the most used hormone for induction of super ovulation in a number of animal species like rat, cattle, pigs and even buffaloes. Although cAMP is the established second messenger for both LH and FSH, many actions of FSH and LH are not mediated by cAMP. Further the dual activity of PMSG in heterologous animal species adds complications in interpreting its actions. Recent work from our laboratory demonstrates that ovarian ascorbic acid is depleted by LH (in corpus luteum) but increased by FSH (in growing follicles). The second messenger status in some of these effects of PMSG (as LH and FSH) is not clear yet. Future research on PMSG is also indicated to clarify some of these questions.

INTRODUCTION

Gonadotropins are chemical messengers regulating physiological functions including maintenance of differentiated functions of gonads (Norman and Litwack, 1997) in all vertebrates. Reproduction is the manifestation of an interaction between nervous and endocrine system. The family of glycoprotein hormones consists of the pituitary Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH) and Chorionic Gonadotropin (CG). The pituitary gonadotropins are found in all vertebrates (mammals and non-mammalian species) while, CG and its receptor areonly reportedin primates (Ascheim and Zondek,1927) and equids (Cole and Hart, 1930; Pierce and Parsons.,1981; Bousefield*et al*., 2006).The pituitary synthesizes and secretes LH, FSH and TSH under the control of hypothalamus while, CG is synthesized mainly by the placental chorionic trophoblastic cells, although there is convincing evidence for supplementary appearance of CG in the pituitary and at incredibly small levels in the serum of normal individuals. CG levels were somewhat elevated in aged (more than 50 years) individuals (Birken *et al*., 1996; Dirnhofer *et al*., 1996). Moreover, it was also reported that hCG levels were receptive to positive feedback viaGnRH *in vivo* and *in vitro* and to negative response via progesterone and estrogen *invivo* (Henke *et al*., 2007).

Corresponding author: Kambadur Muralidhar
Faculty of Life Sciences and *Biotechnology, South Asian University, New Delhi-110021, India.*

Two of the pituitary gonadotropins, Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) regulate testicular and ovarian functions by stimulation of gametogenesis and steroid synthesis in gonads (Catt and Pierce, 1978).Glycoprotein hormones being heterodimeric in natureare composed of a common, species specific alpha (α) subunit, non-covalently coupled with the hormone specific beta (β) subunit. Glycoprotein hormones exert their biological actions byinterrelating with their associated receptors. Just like the hormones, their cognate receptors are also closely associated. LH and CG utilize the same receptor, LHR; FSH binds to FSHR and TSH to TSHR (Segaloff and Ascoli, 1993). Receptors of the gonadotropins belong to the Leucine-rich-repeat-containing G-protein coupled receptor (LGR) subfamily (Hsu *et al*., 1998, 2000).

The LGR subfamily, in turn, belongs to Family A of the G-protein coupled receptor (GPCR) super family. GPCRs transduce extracellular signalsvia its seven-helical Trans membrane (seven TM) domains to stimulate G-protein (Pierce *et al*., 2002). The classical component of Family A is rhodopsin. LGRsare distinct from the non-LGR members of Family A in their ectodomains. While the non-LGR members enclose small extracellular N-terminal peptides and bind small molecules, the LGRs have strangely large ectodomains consisting ofLeucine-rich repeats (LRR) (Wang *et al*., 2013). The GPHR ectodomains consist of 340–420 amino acid residues and bind their large ligands which have molecular weights of about 33 kilo Daltons (kDa).

Upon communication with hormone, the hormone-induced conformational transformation in the cognate receptor which, concurrently transduce the hormone signals down-stream to the in the interior of the objective cell, by putting on a little number of signaling molecules, basically a G-protein heterotrimer, foremost to the dissociation of α- and dimeric βγ-subunits. The α-subunit then triggers stimulation of adenylyl cyclase, results amplification of cAMP, which eventually leads to augmented production of steroids in the case of LH/CG and FSH. Hence forward, the free βγ dimmers conscript GPCR kinases (GRK) to the receptor, which phosphorylate the intracellular loops of the receptor. That in turn, escorts to the conscription of β-arrestin to the receptor. By this way and many other signal cascades (McDonald *et al*., 2006), the hormones coordinate and synchronize the indispensable physiological reactions in its individual tissues.

Placental gonadotropins i.e. CG is the foremost biochemical message from the embryo to the maternal ambience. It is synthesized and secreted by extra embryonic trophoblast at the commencement of pregnancy. It attains peak concentrations during first trimester and is critical for stimulating the corpus luteum to produce Progesterone which is essential for repression of further follicle maturation, thus guarantying embryo implantation and maintenance and continuation of pregnancy (Pierce and Parsons, 1981). Throughout gestation, CG is produced by the placental fetal part and is also critical for male fetal sexual differentiation, as CG arouses the fetal testosterone synthesis in the testicular Leydig cells (Gromoll *et al*., 2000). Information of CG's promotion of angiogenesis validates the assumption that the embryo encourages maternal blood vessel growth via CG for better delivery of nutrients and oxygen and easier liberation of CG and other factors (Herr*et al*., 2007).

Additionally, it was also evidenced that CG is essential for the incursion of cytotrophoblasts into the endometrium during embryo implantation (Carver *et al*., 2003). Investigations of Rull and Laan (2005) established the correlation among persistent miscarriage and low serum level of CG in patients; so it is believed that low serum CG hampers embryo implantation.LH, FSH and human chorionic gonadotropin (hCG) being well characterized and extensively studied among pituitary and placental reproductive hormones.Human menopausal gonadotropin (hMG) and PMSG (eCG) are least characterized with regard to biochemical and physiological properties. Pregnant mare serum gonadotropin is also a member of glycoprotein hormone produced by fetal trophoblast cells in mares and is related to pituitary gonadotropin in both chemical nature and biological activity. PMSG is a unique molecule of special attention because of its FSH and LH hormonal activities in the same molecule.

PMSG

During mare gestation, PMSG is synthesized and secreted by the endometrial cups which come up from the placental trophoblast chorionic girdle cells. In pregnant mare serum, CG emerges from day 40 of conception onwards, enhances until it attains its highest point between days 60 to 70 and drops off until day 130 and then becomes unnoticeable (Wooding *et al*., 2001). In contrast, hCG is noticeable prior at the very beginning of female conception,unlike CG in mares, and drops after a peak to a low, but still measurable level, until parturition.

LH-FSH duality of PMSG is a species–specific phenomenon. It is of tremendous importance for basic agricultural (veterinary) science and research. It has been extensively used in super ovulation programme in farm animals like cattle, sheep goats, camels etc. Significant biological and immunological diversities subsist between endometrial cups derived eCG and PMSG (serum derived) depending on the assay system used for characterization. Assorted studies by Aggarwal *et al* (1980) recommended that the characteristics of eCG can differ depending upon the source of material taken for purification. eCGisolated from endometrial cups had 6% more carbohydrate than PMSG in serum which suggested that the micro-heterogeneity of gonadotropins was found mostly due to differentiation in glycosylation.

It was also known that eCG was found to be twice as active as PMSG in the rat Leydig cell LH bioassay (involving testosterone production). Further,eCG was found to be more potent than PMSG in the equine and calf testes FSH RRAs, but both fractions significantly less potent compared to eFSH (Papkoff,1978). The discrepancies among these eCG preparations may explain variations between stored and secreted forms of the hormone. It might be pertinent that the secretion of variousisoform of eCG may be under the control of endocrine milieu, as per the reports of pituitary gonadotropins.

Immunological studies on PMSG

The haemagglutination inhibition reaction based on hCG detection for the diagnosis of pregnancy in women was reported by Wide and Wide (1963). In this reaction hCG inhibited the agglutination of stable hCG-coated blood cells by hCG antibodies. The same immunological technique was applied by Wide and Wide in 1963 for assay of PMSG in PMS and was applied as a pregnancy detection test in mares. PMS containing elevated level of gonadotropin inhibited the agglutination reaction of PMSG-coated sheep formalinized erythrocytes in the presence of PMSG antibody, while antiserum fromnon-pregnant mares did not gave this reaction. Schams and Papkoff(1972) immunized white New Zealand rabbits with purified PMSG and produced antibody against PMSG. They also characterized the antiserum by agar diffusion (Ouchterlony technique), immune electrophoresis and quantitative precipitin reactions; it was specific for PMSG and did not cross react with hLH and hFSH.

Radio receptor assay measures interlinking competence of hormones to their cognate receptor positions and as such are measured to give a nearby assessment of biological activity. Stewart *et al*(1976) developed and validated the radio receptor assay for assessmentof FSH-like and LH-like activities of PMSG. This method had been used to determine the FSH: LH ratios in the serum of mares at intervals during mare gestation, in endometrial cup secretion, in crude preparations of serum extracts of PMSG, and in the supernatants isolated from cultures of PMSG producing equine trophoblast cells. They also checked immunological relationship among PMSG, its subunits and eFSH. Menzer and Schams (1979) developed a double antibody radioimmunoassay (RIA) for PMSG, for measuring PMSG in cattle blood after exogenous application. They used PMSG antiserum and PMSG for radio iodination.Farmer and Papkoff (1979) developed and

characterized homologous double antibody RIA for PMSG. With this RIA they measured PMSG concentration in PMS and checked immunological relationship among PMSG, its subunits and eLH and eFSH. Bousefield*et al* (1989) used monoclonal antibody raised against eCG and hCGas probes for the topographic analysis of epitopes on the human α-subunit to obtain information on the three dimensional structure of hCGα. Combarnous *(*1992) raised monoclonal antibody against the native hormone and did immunochemical mapping of PMSG using three antibodies – ECG01, E10, and D7 and indicated that, distinct antigenic determinants were found to be located on the eCG and hCG. The selected mAbs represents powerful tool for the study of structure functional relationship of this hormone that possess an inimitable prototype of bioactivity.

Similarly,Bousefield*et al* (1996) used combination of monoclonal antibodies (mAbs) specific for epitopes that resides on free and combined subunits of eCGfor development of immunoradiometric assays (m-IRMAs) and recognition of eCG, eCGα, eCGβ. Their results suggested that eCG andfree subunit production in pregnant mares begins at the beginning of gestation was similar to that observed in pregnant women. These immunoassays specific for either intact hormone or its freesubunits, constitute useful diagnostic tools for investigating reproductive problems in mares. Nell and Gielen (1995) developed monoclonal antibody against PMSG for veterinary application. In India A.K Gupta and Meenakshi Virmani in 2008, claimed development of haemagglutination inhibition assay for eCG detection in serum and to detect pregnancy of mare at any stage (personal communications).

Biological Characteristics of PMSG, the FSH-LH Duality

Based on the morphological characteristics of the *in vivo* ovarian responses induced in rodents by these two hormones, Hisaw and co-workers named the hormones follicle stimulating hormone (FSH) and Luteinizing hormone (LH). The biological behaviors of crude PMSG in the immature female rat look like that of a mixture of FSH and LH, that is, PMSG induces a complete gonadotropic stimulation of the reproductive tract (Hamburger, 1957).It was previously considered that whole pregnant mare serum also possess two separate gonadotropins. In fact, numerous groups of investigators claimed to have fractionated pregnant mare serum into both FSH-like and LH-like components (Frahm and Schneider, 1957). This claim was however, stands contradicted by other investigation. Cole and his colleagues demonstrated that even the fractional precipitations also failed to separate the follicle and luteinizing cell stimulating activities of PMSG. (Pencharz *et al*., 1940).

The assertion of Frahm and Schneider (1957) that PMS could be isolated into FSH and LH components by paper electrophoresis had also been doubted.Several groups of investigators (Raacke*et al*., 1957) held the view that interpretations of the results of Frahm and Schneider (1957), Evans *et al* (1936) were erroneous and could have been correctly explained by the conclusions of Hamburger (1957), who established that the macroscopic effects obtained with PMSG were related to the enormity of the dosages administered. Low dosages of PMSG had an ovarian LH effect.

The *in vivo* activity of PMSG in the male rat had also been comprehensively evaluated. In the intact immature male, PMSGprovokes an augmentation in testicular weight which could be accredited to proliferation of spermatocytes, extension of seminiferous tubules and reasonable magnification of interstitial tissue (Cole *et al*., 1932). The weights of the seminal vesicles and prostate were noticeably augmented there by demonstrating that the hormone has an LH-like effect in the male animal. Similar responses had also been seen in immature male rats which had been hypophysectomized (Cole *et al*., 1932; Raacke*et al*., 1957). Cole *et al* (1940) illustrated that daily administration of 20 IU of PMSG to hypophysectomized male rats for a period of 20 consecutive days resulted in an outstanding augment in accessory sex organ like prostate weight from a control value of 25 mg to a standard weight of 375 mg. This weight represented a 4-fold augmentation in prostate weight over that of an intact rat of the same age. Thus it can be concluded that PMSG also has a strong LH-like effect in immature hypophysectomized male rats. The likelihood that some of the complete gonadotropic effects induced by PMSG were related circuitously to the actions of endogenous gonadotropins was disputed when it was established that the PMSG induced responses in immature, hypophysectomized rats were similar to those obtained with intact animals (Cole *et al*., 1940). Several laboratories had provided proof which suggests that PMSG induces the secretions of endogenous pituitary gonadotropins which then directly make possible the regulation of this particular ovarian event (Sasamoto and Kennen, 1973). Moreover, it had been shown by Nuti*et al* (1975) that a single administration of PMSG (8IU) can transform a 30 day old immature female rat into a reproductively mature animal by inducing the commencement of neuro-endocrine orchestra which controls the function of the reproductive system.In addition, some reports which were available contained conflicting data relating to the comparative amounts of the FSH and LH activities associated with PMSG. In 1967, using the FSH assay of Steelman and Pohley (1953), involving ovarian weight assessment, and the LH assay of Diczfalusy (1954), involving male accessory gland weight assessment, Dorner and Gotz (1967) concluded that PMSG possess greater LH activity than FSH activity (FSH: LH ratio = 0.1) when assayed in terms of the Second International Reference Preparation of human menopausal gonadotropin (2nd IRP-HMG).

Combarnous *et al.* (1978) reported higher affinity of PMSG for LH receptor than FSH receptor of porcine testis. These studies were the first to consider which activity of PMSG is dominant, the FSH or LH activity. However, the disparities may be correlated to the origin of the PMSG and/or, the techniques used for purifying or assaying the hormonal activity. A highly purified preparation of PMSG which had been extensively characterized was assayed *in vivo* by the Steelman-Pohley FSH assay (1953) and the Parlow (1961) OAAD LH assay (Papkoff *et al*., 1978). This preparation assayed at 75.9 times NIH-FSHS10 in the FSH assay and 2.44 times NIH-LH-S18 in the LH assay. Both of these values are comparable to those obtained with highly purified preparations of FSH (Braselton and McShan, 1970) and LH (Ward *et al*., 1959; Papkoff *et al*., 1965). These results obtained from *in vivo* assays in the rat suggest that PMSG expresses comparable levels of both FSH and LH activities indicating a lack of predominance of either activity.

Biological activity and role of PMSG in equids

Cole and his colleagues first suggested that the dual gonadotropin activity of PMSG wasdependent on a single substance because they were unable to separate FSH and LH activities in PMSG by fractional precipitations. After these crude preparation studies many more like Stewart *et al*(1976), Papkoff *et al* (1967), Combarnous *et al* (1978), Moyle *et al* (1978), and Christakos and Bahl(1979) used purified preparation of PMSG and suggested that it's the single molecule having both FSH and LH activity. But PMSG acts primarily as lutropin of pregnancy was firstly suggested by its discoverer Cole (Cole *et al*, 1931).Further support for the theory that endogenous PMSG might function primarily as a lutropin in the mare was provided by the work of Stewart *et al.* (1976), which assessed the*in vitro* FSH: LH activity ratio of PMSG derived from various sources.

These workers demonstrated that culture of equine trophoblast cells secreted a PMSG having an FSH: LH activity ratio which was one half of serum-derived PMSG. One interpretation of this finding was that freshly secreted PMSG acts primarily as an LH; FSH activity being acquired only after some type of modification of the molecule occurring in the circulatory system of the mare. Ward *et al*. (1982) by radio ligand binding assay technique used both horse testis homogenate and rat testis homogenates, based on % inhibition proved that PMSG is primarily lutropin in equine group and both lutropin and follitropin in non equids. They proved that the demonstration of PMSG lutropin-follitropin duality is a species specific phenomenon.They also reported that the upon reassociation of PMSG α and PMSG β subunits, the lutropin activity was restored to a greater extent than the follitropin activity, suggesting that PMSG may be a heterogeneous population of modified and unmodified molecules or that conformational requirements for the expression of follitropin by a reassociated complex of PMSG subunits are more extensive than those necessary for the expressions of lutropin activity. Mare CG bound to putative LH receptors in equine testis (Papkoff *et al*1978). However, this happened at about one-tenth or less the affinity of horse LH binding (Papkoff *et al.,* 1978). The reduced receptor binding activity of PMSG in mare may be due to the carbohydrate content of horse CG somehow inhibits its binding to the horse LH receptor.

An alternate view is that the receptor population of the horse gonad may be heterogeneous, and separate binding sites of different affinities may be capable of discriminating between eCG and eLH. In support of this view, Sairam *et al.*(1988)have shown that the ovine testicular LH receptor has separate binding sites which can distinguish LH from hCG or eCG. eCG does not bind to FSH receptors in mare follicles (Combarnous,1992) or testis (Moore and Ward, 1980), suggesting that CG is primarily an LH-like hormone in the horse. It is of interest that horse CG binds to donkey FSH receptors with similar affinity to its attachment to donkey LH receptors (Stewart and Allen, 1979), a finding that has been interpreted to indicate that eCG may have FSH activity in the donkey (Stewart and Allen, 1979). The biological activity of donkey CG had been much less studied, and the homology between donkey CG and donkey LH in primary structure had not been confirmed. However, donkey LH binds to equine testis preparations with about 10% of the activity of the horse LH (Matterai*et al*., 1987). Horse CG is believed to be constitutively expressed by the trophoblastic cells until the endometrial cups degenerate. The role of CG in equine gestation is remains obscure.It is believed to act as an LH-like hormone to induce supplementary ovulation and/or luteinization of follicles in the mare. It has not been established whether CG or the accessory corpora lutea are necessary for successful horse pregnancy. Furthermore, Spincemaille *et a1*.(1975) had reported that mare pregnancy proceeds normally in the absence of detectable levels of PMSG. These workers were unable to detect any PMSG in the serum of one mare up to gestation day 70. In addition, sacrifice of the animal on gestational day 70 revealed the absence of endometrial cups in the uterus and the lack of PMSG activity in extracts of endometrial tissue. Macroscopically the conceptus appeared normal.Moreover, in the same study another mare, in which no PMSG was detected, gave birth after a normal pregnancy. It may serve as a redundant system to assure that there is sufficient secretion of the primary corpus luteum to maintain pregnancy until the placenta assumes its role as the principal steroidogenic organ of gestation.

Biological activity and actions of PMSG in non-equids

One of the peculiar properties of PMSG is its duality of function in non equids which was demonstrated earlier by Cole *et al.* (1946). This can be demonstrated in the conventional Steelman–Pohley bioassay (1953) for FSH, OAAD for LH (Parlow, 1961) and several *in vitro* and *in vivo* assays. Studies by Moore and Ward(1980) and Stewart *et al.* (1976) demonstrated that with rat Sertoli cell receptor PMSGacts as FSH and with Leydig cell receptor it acts as LH. Farmer and Papkoff (1979) demonstrated that PMSG acts like FSH in rat seminiferous tubules because it stimulates cAMP level. Moyle *et al.* (1978)demonstrated FSH action of PMSG by its capacity of estrogen production in cultured granulose cell from immature rat ovaries. Desialiation of PMSG resulted in a reduction of slope and potency of this response suggesting that sialic acid may be important for the interaction of PMSG to Sertoli cell receptor.

Folliculogeneticpotential, which is an indication FSH andLHaction of PMSG, had been assessed by determination of number of ovulations in immature rats (Martinuk*et al*., 1990). Murphy *et al* (1984) removed sialic acid by neuraminidase digestion and this increased the LH activity of PMSG on the Leydig cells assay in a manner related to the quantity of sialic acid removed and, LH activity increases fivefold when sialic acid is completely removed (Aggarwal and Papkoff, 1985). There is a concept regarding the PMSG and is that it has a constant ratio between FSH and LH activity in equine CG (Stewart *et al*, 1977). It was clearly demonstrated that PMSG is a heterogeneous group of molecules and its carbohydrate content can vary depending upon source of CG i.e. cups/serum and also preparation ofCG pooled fromgroups of mares or between mares and between stages of gestation. Because of this heterogeneity, equine CG can vary greatly in its biological activity (Gonzalez-Menico *et al.,* 1978).The remarkable property of PMSG that has been exploited worldwide is its capacity to express FSH like activity in non equids. The biological basis for this phenomenon remains incompletely understood. Partially purified crude preparations of PMSG are the least expensive and most readily available means of induction of

folliculogenesis in domestic and laboratory animal sciences (Stewart and Allen, 1995).Donrov*et al.* (1998) used crude PMSG for injecting to Mongolian native ewes for synchronization of Estrous. Estrous was induced in these Mongolian ewes. Peter and Menzes (1984) studied the effect of super-ovulatory doses of PMSG and hCG on ovulation, advancement of ovulation, subsequent embryo development and implantation in golden hamster. Bravo *et al* in 1995 administrated PMSG in Llama for induction of growth and development of multiple ovarian follicles. PMSG has been routinely used worldwide forsuper ovulation protocol in dairy goats (Baril *et al*., 1996), sheep (Martemucci and D'Alessandro, 2011), cow (Gonzalez *et al*., 1994) and buffalo (Techakumphu*et al*., 2000).

In the early days of embryo transfer, PMSG was administered to induce super ovulation in donor animals. Its prolonged circulatory half-life confers the benefits of super ovulation, but the same quality limits its application also, as it has the tendency to hyper activate the ovary resulting multiple unovulated follicles and reduced yield of viable embryo (Bevers *et al*., 1989). Administration of higher dose of PMSG resulted higher number of ovulations, produced fewer number of transferable embryos (Gonzalez*et al*., 1994). The reduced embryo quality had also been attributed to the LH activity of PMSG, as it may cause precocious resumption of meiosis in the oocyte, resulting in aneuploidy and other problems of early development. However, the repeated use of PMSG administration for ovulation induction is generally followed by a decrease in fertility from 60% to 40%. This phenomenon canbeen explained by unwanted immunological responses. Moreover, it was also reported that ovarian refractoriness to subsequent PMSG stimulation had been associated with the presence of PMSG binding immunoglobulin in many species such as the rhesus monkey (Bavister *et al.,* 1986), cat (Swanson *et al*.,1996), cow(Jainudeen *et al*., 1960), alpine goats and dairy goats (Herve *et al.,* 2004) and sheep (Pigon *et al*., 1960).

Some advances had been made in understanding of dual biological activities of PMSG but further advanced and multifaceted investigation is needed to confirm and extension of the existing findings. Existing enigma of PMSG dual action restricted its usefulness in inducing folliculogenesis, promoting puberty, reversing anestrous and inducing super ovulation for embryo transfer technology in number of species. Better understanding of surface topology of PMSG for dual action can permit investigator to develop a best super ovulation protocol as effectiveness of a single application of PMSG, make these hormones valuable for breeding and research programme as compared to other hormones which have shorter half life and may require repetitive administration.

Bioassays for PMSG

The conventional in vivo assays for PMSG was the one developed several years ago by Cole and Erway (1941) in which dose dependent effect of PMSG on ovarian weight in prepubertal 23 days old rat was measured. This bioassay was reasonable and is characteristically exploited by investigators and manufacturers for evaluation of crude and enriched PMSG preparations. In this bioassay immature rats (of 23 days old) of each groups were administered single s.c injection of one dosage of PMSG prepared in saline and 48 hours later, the animal were sacrificed. It was reported in this paper that the uterine weight augmentation was found to be sturdier to a great extent and more sensitive than ovarian augmentation. On the other hand, uterine weight augmentation parameter was not selected by investigators as they considered ovarian augmentation a more straight reaction in spite of its higher response threshold.

Very recently, Lecompte*et al.* (2010) reassessed the method of Cole and Erway and utilized their information of uterine weight augmentation to develop rat uterotropic bioassay for PMSG, hCG, pituitary LHs and FHSs. It was reported in this assay that uterine weight augmentation was higher and observed at the concentration of PMSG which are approximately 15-30 fold lower than ovarian augmentation. Furthermore, uterine augmentation reaction curve slope was found to a great extent steeper than that of the ovarian augmentation response curve. Authors claimed high reproducibility and sturdiness of the uterotropic assay for PMSG. Not only that they considered their method easier and quicker to perform than methods of Cole and Erway as taking out of a single well-built uterus was found to be uncomplicated than the dissection of two delicate ovaries.

While studying the OAAD activity of LH in peudopregnant immature female rats, it was observed that this action was mediated by cAMP and that this activity is Actinomycin D and Cyclohexiimide insensitive (Arora *et al*, 2012). It was also observed that there was no increase in specific activity of ovarian L-Gulonic acid dehydrogenase (L-GuDH) activity (MPhil Thesis, 2012). Subsequent studies showed that PMSG administration into immature female rats actually results in increase on ovarian ascorbic acid. Of course this is FSH like action on follicular tissue while LH action was observed on corpus luteum. As ascorbic acid inhibits the same enzyme *in vitro* (Vineet Sharma *et al*, 2013), it was interpreted that high levels of ascorbic acid keep the enzyme inhibited *in vivo* and by some unknown mechanism when LH causes a depletion in ovarian ascorbic acid, the enzyme is released from inhibition.

Unlike in the case of LH action, FSH action in increasing ovarian ascorbic acid content was shown to result in simultaneous increase in specific activity of L-GuDH (Shah Saddad Hussain *et al*-unpublished results). Current thinking is that L-GuDH may not have a regulatory role in regulating ovarian ascorbic acid levels. Microarray analysis also indicated that the lactonase gene expression increases significantly when PMSG increases ascorbic acid content in ovaries (Shah Saddad Hussain *et al*-unpublished results). Our recent work also has shown that antiserum to eLH is capable of neutralizing both the LH and FSH like activities of PMSG in rats and mice (Nikki Kumari *et al*-unpublished results).

Comments

PMSG like other glycoprotein hormone exhibits micro heterogeneity due to minor chemicalvariation in their carbohydrate moieties as well as in their polypeptide chain. In some cases these differences are believed to results in changes in biological activities however, these multiple isoform of a given hormone or subunits are always encoded by single gene and their differences results from co-translational or post-translational events. Despite the availability of considerable amount of information on the structure of PMSG and other glycoprotein

hormone, there is need of rigorous analysis and the assignment of specific structures responsible for particular function. Structural analysis of PMSG and its subunits by high field and nuclear magnetic resonance X-ray diffraction data will enrich us with detailed structural functional analysis of this hormone. Crystallization of glycosylated and deglycosylated forms of hCG is a major achievement. Like hCG, PMSG crystallization will lead to an accurate three dimensional structures of this protein and will enrich us with detailed structural analysis of this particular hormone.

PMSG has been recognized as pregnancy specific hormone, but no detailed studies on its biology and its differential role in gestational progress has been undertaken as there are other hormone in equids like eLH and eFSH doing same major function. PMSG is available in limited quantities from commercial sources which are giving multiple bands in SDS-PAGE shows its impurity so; there is need of good purification protocol with few chromatographic steps to avoid loss of hormone activity with larger purification steps. Mechanism of action of PMSG in non-heterologous organism like rat, mice, goat, and sheep is not known which can also be resolved by measuring second messenger and studying hormone receptor interaction. There is need of good sandwich ELISA to measure quantity of PMSG in PMS. Development of a super ovulation protocol without its ovarian cyst inducing property using PMSG will help in breeding programmes. PMSG is unique among all glycoprotein hormones because it has both LH and FSH activities contained within the same molecule. It is conceivable that these activities reside in different parts of the same molecule. Thus detailed structural analysis and characterization of PMSG should enable us to delineate this mystery. Combination of structural-functional guided approaches and good super ovulation protocol of PMSG without its ovarian cyst inducing properties would help to enrich the therapeutics related to infertility and assisted reproductive technology. However, there are still enigmas about the dual biological activity of PMSG.

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REFERENCES

Aggarwal, B. and B, Papkoff, H.1985.Plasma clearance and tissue uptake of native and desialylated equine gonadotropins.*Domest Anim Endocrinol.,* 2,173.

Aggarwal, B., Farmer, S. W., Papkoff, H. and Seidel, G. E. Jr. 1980.Biochemical properties of equine chorionic gonadotropin from two different pools of pregnant mare serum.*Biol. Reprod,* 23, 570-576.

Arora, T. Rhisita Nath, Nidhi Vashistha, Nikki Kumari, Shah Saddad Hussain and Kambadur Muralidhar, 2012. Mechanistic investigations of the dual activity of Gonadotropins using target tissue cAMP level as a response parameter. *World J. Life Sci. Med Res.,* 2 (3) 11-19.

Ascheim, S. and Zondek, B. 1927.The hormone of the anterior lobe of the hypothesis and ovarial hormone in the urine of pregnant women.*Kin.Wschr*. 6, 1322. (not seen in the original)

Baril, G., Remy, B., Leboeuf, B., Beckers, J.F. andSaumande, J. 1996. Synchronization of estrus in goats: the relationship between ECG binding in plasma, time of occurrence of estrus and fertility following artificial insemination. *Theriogenology*, 45, 1553–1559.

Bavister, B.D, Dees, C. and Schultz, R.D. 1986. Refractoriness of rhesus monkeys to repeated ovarian stimulation by exogenous gonadotropins is caused by no precipitating antibodies. *Am. J. Reprod Immunol. Microbiol.,*11, 11–16.

Bevers, M.M., Dielman, S.J., van Tol, H.T., Blankenstein, D.M, van den Broek, J. 1989. Changes in pulsatile secretion patterns of LH, FSH, progesterone, androstenedione and oestradiol in cows after superovulation with PMSG.*J. Reprod Fertil*.,87, 745- 754.

Birken, S., Maydelman, Y., Gawinowicz, M.A., Pound, A., Liu, Y. and Hartree, A.S. 1996. Isolation and characterization of human pituitary chorionic gonadotropin.*Endocrinology*, 137, 1402–1411.

Bousfield, G. R., Li. J. and Ward, D. N. 2006.Gonadotropins: Chemistry and Biosynthesis. In Knobil and Neill's *The Physiology of Reproduction, 3rd edition, Neill, J.D. Ed.,Elsevier* ,Chapter 30, Vol.1,1581-1634..

Bousfield, G.R, Liu, W.K. and Ward, D.N. 1989.Effects of removal of carboxy terminal extension from equine luteinizing hormone (LH) beta subunit and follicle-stimulating hormone receptor binding activities and LH steroidogenic activity in rat testicular Leydig cells.*Endocrinology*. 124, 379-387.

Bousfield, G.R., Butnev, V.Y., Gotschall, R.R., Baker, V.L. and Moore, W. T. 1996.Structural features of mammalian gonadotropin.*Mol Cell Endocrinol*., 125, 3-19.

Braselton, W. E., Jr. and McShan, W. H. 1970.Purification and properties of follicle-stimulating and luteinizing hormones from horse pituitary glands.*Archs.Biochem.Biophys*. 139, 45-58.

Carver, J., Martin, K., Spyropoulou, I., Barlow, D., Sargent, I. andMardon, H. 2003.An *in vitro* model for stromal invasion during implantation of the human blastocyst.*Hum. Reprod.* 18, 283–290.

Catt, K. J., and Pierce, G. 1978.In Reproduction endocrinology, ed.S.S.C.*Yen, R.B, Jaffe. Philadelphia, Saunders*. 34-62..

Christakos, S. and Bahl, O.P. 1979. Pregnant mare serum gonadotropin: purification and physicochemical, biological, and immunological characterization. *J. Biol. Chem.,* 254, 4253

Cole, H. H. and Erway, J. 1941.48-hour assay test for equine gonadotropin with results expressed in international units.*Endocrinology*, 29, 514 –519.

Cole, H. H. and Hart, G.H. 1930.The potency of blood serum of mares in progressive stages of pregnancy in effect in the sexual maturity of the immature rat. *Am. J. Physiology,*93, 57-68.

Cole, H. H., Pencharz, R. L. and Goss, H. 1940.On the biological properties of highly purified gonadotropin from pregnant mare serum.*Endocrinology*, 27, 548-553.

Cole, H.H., Guilbert, H. R. and Goss, H. 1932.Further considerations of the properties of the gonad-stimulating principle of mare serum.*Am. J. Physiol*., 102, 227-240.

Cole, H.H., Howell, C. E. and Hart, G. H. 1931.The changes occurring in the ovary of the mare during pregnancy.*Anat. Rec*., 49, 199-209.

Cole, H.H., Penchars, R.L. and Goss, H. 1946.On the

biological properties of highly purified gonadotropin from pregnant mare serum. *Endocrinology,* 27, 548.

Combarnous, Y. 1992.Molecular basis of the specificity of binding of glycoprotein hormones to their receptors.*Endocr. Rev*., 13, 670–691.

Combarnous, Y., Hennen, G. andKetelsleger, J. M. 1978. Pregnant mare serum gonadotropin exhibits higher affinity for lutropin than follitropin receptors of porcine testis. *FEBS Letters,* 90, 65-68.

Diczfalusy, E. 1954.An improved method for the bioassay of chorionic gonadotropin.*Actaendocrinol.Copenh.* 17, 58-73.

Dirnhofer, S., Hermann, M., Hittmair, A., Hoermann, R., Kapelari, K. and Berger, P. 1996.Expression of the human chorionic gonadotropin-beta gene cluster in human pituitaries and alternate use of exon 1. *J. Clin. Endocrinol.Metab*.,81, 4212–4217.

Donrov, Ts.,Batsaihan, D. and Ley, W.B. 1998. Gonadotropin extraction from pregnant mares serum and effect of PMSG preparation on the fertility of Mongolian native ewes, *Small Ruminant Research,* 28, 61–66.

Dorner, G. and Gotz, H.G. 1967. The follicle-stimulating and interstitial cell-stimulating activities of pregnant mare serum gonadotropin compared with those of other gonadotropins *J.Endocrinol.,* 39(4), 609-10.

Evans, H. M., Korpi, K., Simpson, M.E. and Pencharz, R. I.1936.On the separation of the interstitial cell-stimulating, luteinizing and follicle stimulating fractions in the protein pituitary gonadotropin complex. Univ. *Calif. Publ. in Anat*. 1, 255-274.

Farmer, S.W. and Papkoff, H. 1979. Immunochemical studies with pregnant mare serum gonadotropin. *Biol Reprod,* 21, 425–31.

Frahm, H. and Schneider, W.G. 1957.Papierelecktrophoretischeautspaltung gonadotropic substanzen.*ActaEndocrinol*.,24, 106-112. (not seen in the original)

Gonzalez, A., Wang, H., Carruthers, T.D., Murphy, B.D. and Mapletoft, R.J. 1994. Superovulation in the cow with pregnant mare serum gonadotropin: effects of dose and antipregnant mare serum gonadotropin serum. *Can. Vet. J*., 35, 158-162.

Gonzalez-Mencio, F., Manns, J. and Murphy, B.D. 1978.FSH and LH activity of PMSG from mares at different stages of gestation.*Anim. Reprod Sci.,*1, 137.

Gromoll, J., Eiholzer, U., Nieschlag, E. andSimoni, M. 2000. Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. *J. Clin. Endocrinol.Metab.,*85, 2281–2286.

Hamburger, C. 1957.The assay of gonadotropin hormones.Asurvey.*ActaEndocrinol.(Suppl).,* 31, 59-74.

Henke, A., Luetjens, C.M., Simoni, M. and Gromoll, J.2007. Chorionic gonadotropin beta subunit gene expression in themarmoset pituitary is controlled by steroidogenic factor 1 (SF1), early growth response protein 1 (Egr1) and pituitary homeobox factor 1 (Pitx1). *Endocrinology*, 148 (12), 6062–6072.

Herr, F., Baal, N., Reisinger, K., Lorenz, A., McKinnon, T., Preissner, K.T. and Zygmunt, M. 2007.hCG in the regulation of placental angiogenesis. Results of an in vitro study.*Placenta (Suppl. A*). 28, S85–S93.

Herve, V., Roy, F., Bertin, J., Guillou, F. andMaurel, M.C. 2004.Anti equine chorionic gonadotropin (eCG) antibodies generated in goats treated with eCG for the induction of ovulation modulate the LH and FSH bioactivities of eCG differently. *Endocrinology,* 145 (1), 294-303.

Hsu, S.Y., Kudo, M., Chen, T., Nakabayashi, K., Bhalla, A., van der Spek, P.J., van Duin, M. andHsueh, A.J. 2000. The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling mechanism for LGR7. *Mol. Endocrinol.,* 14, 1257–1271.

Hsu, S.Y., Liang, S.G. andHsueh, A.J. 1998. Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane region.*Mol. Endocrinol.,* 12, 1830–1845.

Lecompte, F., Harbebya, E., Cahoreau, C., Kletta, D. and Combarnous, Y.2010.Use of the immature rat uterotrophic assay for specific measurements of chorionic gonadotropins and follicle-stimulating hormones *in vivo* bioactivities.*Theriogenology,* 74, 756–764.

Martemucci, G., D'Alessandro, A.G. (2011) Synchronization of oestrus and ovulation by short time combined FGA, PGF (2alpha), GnRH, eCG treatments for natural service or AI fixed-time. *Anim. Reprod. Sci.,* 123, 32-39.

Martinuk, S.D., Rajkumar,K. and Murphy, B.D. 1990..Comparison of circulating and placental forms of equine chorionic gonadotropin.*Domest. Anim.Endocrinol*., 423-428.

Matteri, R.L., Baldwin, D.M., Lasley, B.L. and Papkoff, H. 1987. Biological and immunological properties of zebra pituitary gonadotropins: comparison with horse and donkey gonadotropins. *Biol. Reprod.,* 36, 1134.

McDonald, C.A., Millena, A.C., Reddy, S., Finlay, S., Vizcarra, J., Khan, S.A. and Davis, J.S. 2006. Follicle-stimulating hormone-induced aromatase in immature rat Sertoli cells requires an active phosphatidylinositol 3-kinase pathway and is inhibited via the mitogen-activated protein kinase signaling pathway. *Mol. Endocrinol*., 20, 608–618.

Menzer, C. and Schams, D. 1979. Radioimmunoassay for PMSG and its application to in-vivo studies.*J. Reprod,Fertil*.,55, 339–45.

Moore, Jr. W.T. and Ward, D.N. 1980. Pregnant mare serum gonadotropin: rapid chromatographic procedures for the purification of intact hormone and isolation of subunits. .*J Biol. Chem.,*255, 6923-6929.

Moore, Jr. W.T. and Ward, D.N. 1980. Pregnant mare serum gonadotropin: an *in vitro* characterization of the luteotropin-follitropin dual activity. *J. Biol. Chem*., 250, 6930-6936.

Moyle, W.R., Erickson, G., Bahl, O.P., Christakos, S. andGutowski, J. 1978.Action of PMSG and asialo-PMSG on rat Leydig and granulosa cells.*Am. J. Physiol.,* 235,18.

Murphy, B.D., Mapletoft, R.J., Manns, J. and Humphrey, W.D. 1984. Variability in gonadotropin preparations as a factor in the superovulatory response. *Theriogenology,* 21, 117.

Nell, T. andGielen, J.1995.The development of a monoclonal antibody against PMSG for veterinary application. *Livest Prod. Sci*., 42(2), 223-228.

Norman, A.W. and Litwack, G. 2001.Hormones :*3rd edition Academic press Elsevier*.

Nuti, K. M., Sridharan, and Meyer, R. K. 1975.Reproductive biology of PMSG-primed immature female rats.*Biol, of*

*Reprod.*13, 38-44.

Papkoff, H.1978. Relationship of PMSG to the pituitary gonadotrophins. In: Sreenan JM (ed) Control of Reproduction in the Cow. *MartinusNijhoff, The Hague*. 73.

Papkoff, H., Bewley, T.A. and Ramachandran, J. 1978.Physicochemical and biological characterizations of pregnant mare serum gonadotropin and its subunits.*Biochim Biophys Acta.*532, 185.

Papkoff, H., Gospodarowicz, D. and Li, C. H. 1967.Purification and properties of ovine follicle stimulating hormone.*Arch.Biochem.Biophys*.,120, 434-439.

Papkoff, H., Gospodarowicz, D., Condiotti, A. and Li, C. H. 1965. Preparation of ovine interstitial cell-stimulating hormone in high yield.*Arch. Biochem. Biophys.,* Ill, 431-438.

Parlow, A.F. 1961. Bio-assay of pituitary luteinizing hormone by depletion of ovarian ascorbic acid.*In: AA Albert, CC Thomas, editors. Human Pituitary Gonadotropins. Springfield (USA*. 300.

Pencharz, R. L., Cole, H. H. and Goss, H. 1940. Response of hypophysectomized rats to highly purified extracts of pregnant mare serum. *Proc. Soc. Exptl. Biol. Med*. 43, 932-933.

Peter, J. and Menzes, J. 1984. Effect of super-ovulatory doses of pregnant mare serum gonadotropin and human chorionic gonadotropin in blastocyst implantation in golden hamsters.*Journal of Biosciences*, 6(S2),75.

Pierce, J.G. and Parsons, T.F. 1981. Glycoprotein hormones: structure and function. *Ann Rev Biochem.,* 50, 465-95.

Pierce, K.L., Premont, R.T. andLefkowitz, R.J. 2002. Seven-transmembranereceptors.*Nat. Rev. Mol. Cell Biol*., 3, 639–650.

Pigon, H., Clegg, M.T. and Cole, H.H. 1960.The formation of antigonadotropin in sheep and its effect on the endocrines and reproductive system.*Acta.Endocrinol.,*35, 253–260.

Raacke, I. D., Lostroh, A. J., Boda, J. M. and Li, C. H. 1957.Some aspects of the characterization of pregnant mare serum gonadotropin.*Acta.Endocrinol,* 26, 377-387.

Rull, K. and Laan, M.2005.Expression of beta-subunit of HCG genes during normal and failed pregnancy.*Hum. Reprod*., 20, 3360–3368.

Sairam, M.R., Yarney, T.A., Bhargavi, G.N. and Sanford, L.M. 1988. Conformational restrictions of the sheep testicular receptor discriminates pituitary lutropin and placental gonadotropins. *J. Biol. Chem*., 263, 3706.

Sasamoto, S. and Kennen, A. L.1973. Endogenous gonadotropin requirements for follicular growth maintenance in immature rats pretreated with PMS. *Endocrinology*.3, 292-296.

Schams, D. and Papkoff, H. 1972.Chemical and immunochemical studies on pregnant mare serum gonadotropin.*Biochim.Biophys.Acta*, 263, 139-148.

Segaloff, D.L. and Ascoli, M. 1993. The lutropin/choriogonadotropin receptor: 4 years later. *Endocr. Rev*., 14, 324–347.

Spincemaille, J., Bouters, R., Vandeplassche, M., Bonte, P. (1975) Some aspects of endometrial cup formation and PMSG production. *J. Reprod.Fertil., [Suppl]*.23, 415.

Steelman, S. L. and Pohley, F. M. 1953. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology,* 53, 604-16.

Stewart, F. and Allen, W.R.1979.The binding of FSH, LH and PMSG to equine gonadal tissues.*J. Reprod. Fertil.,[Suppl]*.27, 431.

Stewart, F. and Allen, W.R. 1995.Comparative aspects of the evolution and function of the chorionic gonadotropins.*Reprod. Domestic Anim.,* 30, 231–239.

Stewart, F., Allen, W.R. and Moor, R.M. 1976. Pregnant mare serum gonadotrophin: ratio of follicle-stimulating hormone and luteinizing hormone activities measured by radioreceptor assay. *J. Endocrinol.,*71:471– 82.

Stewart, F., Allen, W.R. and Moor, R.M. 1977. Influence of fetal genotype on the follicle-stimulating hormone: luteinizing hormone ratio of pregnant mare serum gonadotropin.*J.Endocrinol.,* 73, 419.

Swanson, W.F., Roth, T.L., Graham, K., Horohov, D.W. and Godke, R.A.1996.Kinetics of the humoral immune response to multiple treatments with exogenous gonadotropins and relation to ovarian responsiveness in domestic cats.*Am. J. Vet. Res*., 57, 302–307.

Techakumphu, M., Lohachit, C., Tantasuparak, I.W., lntaramongkol, I.C.S. and Intaramongkof, S. 2000. Ovarian responses and oocyte recovery in prepubertal swamp buffalo (bubalus bubalis) calves after FSH or PMSG treatment. *Theriogenology*, 54, 305-312.

Vineet Sharma, Shah Saddad Hussain, Pooja, Sonia, K. Nirmala and KambadurMuralidhar 2013. Buffalo Kidney L-Gulonate Dehydrogenase: Isolation and Kinetic Characterization*World J. Life Sc. Med Res.,*3(1) 8-14.

Wang, D., Huang, B., Zhang, S., Yu, X., Wu, W. and Wang, X. 2013.Structural basis for Rspondin recognition by LGR4/5/6 receptors.*Genes Dev.,* 27, 1339–1344.

Ward, D.N., McGregor, R.F. and Griffin, A.C. 1959.Chromatography of luteinizing hormone from sheep pituitary glands.*Biochim.Biophys.Acta*.32, 305-314.

Ward, D.N., Moore, Jr., W.T. and Burleigh, B.D. 1982.Structural studies on equine chorionic gonadotropin.*J. Protein Chem.,* 1, 263.

Wide, M.and Wide, L.1963. Diagnosis of pregnancy in mares by an immunological method. *Nature.* 198, 1017-8.

Wooding, F.B.P., Morgan, G., Fowden, A.L. and Allen,W.R. 2001.A structural and immunological study of chorionic gonadotropin production by equine trophoblast girdle and cup cells.*Placenta*., 22, 749–767.

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