A CRITICAL REVIEW OF GONADOTROPIC ACTIVITY OF PREGNANT MARE SERUM GONADOTROPIN

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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 is synthesized mainly by the placenta in mare/horse but exhibits both LH and Follitropin (FSH) like activities in heterologous animal species. Studies have revealed that PMSG acts like Luteinizing Hormone (LH) in horse/mare but exhibits both LH and Folliotropin (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.

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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 Folliotropin (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 reported in primates (Ascheim and Zondek,1927) and equids (Cole and Hart, 1930; Pierce and Parsons.,1981; Bousefieldet 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).

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 nature are 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 by interacting 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-repeats-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 signals via seven-helical Trans membrane (seven TM) domains to stimulate G-protein (Pierce et al., 2002). The classical component of Family A is rhodopsin. LGRs are 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 of Leucine-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 adenyl 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 regulates maternal blood vessel growth via CG for better delivery of nutrients and oxygen and easier liberation of CG and other factors (Herret 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. eCG isolated 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 various isoform 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 from non-pregnant mares did not give 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 assessment of 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 radioiodination. 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 hCG as probes for the topographic analysis of epitopes on the human α-subunit to obtain information on the three dimensional structure of hCG. Comburnous (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 eCG for development of immunoradiometric assays (m-IRMA) and recognition of eCG, eCGa, eCGb. Their results suggested that eCG and free 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 posses 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, PMSG provokes 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; Raidke et al., 1957). Cole et al (1940) illustrated that daily administration of 2 IU of PMSG to hypophysectomized male rats for a period of 20 consecutive days resulted in an outstanding augment in accessory sex organ 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 Kenny, 1973). Moreover, it had been shown by Nutiet 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).

Comburnous 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 (Brasleton 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 was dependent 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 Bahli (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 their 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 (Mattera et al., 1987). Horse CG is believed to be constitutively expressed by the trophectodermal cells until the endometrial cups degenerate. The role of CG in equine gestation 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 al. (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, OAAAD 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 PMSG acts 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. Desialilation 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.

Folliculogenetic potential, which is an indication FSH and LH action 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 of CG pooled from groups 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
foliculogenesis in domestic and laboratory animal sciences (Stewart and Allen, 1995). Donrovet 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 for super ovulation protocol in dairy goats (Baril et al., 1996), sheep (Martemucci and D‘Alessandro, 2011), cow (Gonzalez et al., 1994) and buffalo (Techakumphuet 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 can be 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 pseudopregnant immature female rats, it was observed that this action was mediated by cAMP and that this activity is Activinogenin D and Cycloheximide 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 chemical variation 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. 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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