

Crude hMG Product from the Urine of Postmenopausal Women in 30% Polyvinylpyrrolidone (PVP) and Propylene Glycol (PG) Toward Histological Changes in Rat

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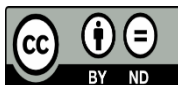


Keywords:

Crude hMG, Postmenopausal Women, 30% Polyvinylpyrrolidone, Propylene Glycol, Histological Changes.

ABSTRACT

Research has produced crude hMG products from the urine of postmenopausal women as an alternative hormone to glycoproteins. Urine hMG crude isolated from local Indonesian women with determination in every 75 IU divided the dose up to 10 IU each dissolved in sterile aquabidest (T0) Observations were made at the intra peritoneal injection site on 30 female white rats, with every 10 females given 1 male. After 48 hours of injection of 10 IU hMG, 10 IU hCG chorulon was injected and waited for 17 hours, then surgery was performed. Observations of histological changes in the peritoneum, kidney, liver and ovaries were divided into groups T0 (10 animals), T1 (10 animals) and T2 (10 animals). No histologic changes were found on the peritoneal surface, ren, liver as a result of injection of hMG 10 IU hMG (T0) Crude hMG dissolved in 30% Polyvinylpyrrolidone (PVP) T1 or Crude hMG in soluble propylene glycol (PG) T2 which is the real purpose of administration of PVP Solvent and PG is to obtain a single dose so that the results of gonadotropin activity are obtained for ease of use in experimental animals $P > 0.05$, meaning that even though 10 IU hMG was used it did not cause histological changes in the tissue studied. However, in white mouse tissue, intraperitoneal injection of hMG 10 IU in T0, T1 and T2 caused changes and growth of follicles on the surface of the ovary until ovulation occurred $P > 0.05$.



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1. INTRODUCTION

Natural urine extracts from menopausal gonadotropin therapy are still used for human infertility or animals. That is, hMG is not species-specific. Gonadotropins or glycoprotein hormones are protein hormones secreted by vertebrate anterior pituitary gonadotropin cells and include the mammalian hormones FSH and LH [18-20]. These hormones are at the heart of the complex endocrine system that regulates sexual development and

reproductive function. LH and FSH are heterodimers composed of two peptide chains with approximately the same alpha chain (about 100 amino acids long), an alpha chain and a beta chain, and the beta chain provides specificity for receptor interaction [43- 46]. The steady development of knowledge about animal and human reproductive processes has led to the identification of higher centers that regulate the dynamics of ovarian function and the discovery of follicle-stimulating hormone. As the mechanism of action of these hormones became more and more understood, they were used in the treatment of infertility in the early 1930s. Hormonal extracts were originally made from animal pituitary and pregnant horse serum, and pregnant human pituitary, placenta, and urine. Due to the difficulty and cost of producing FSHLH, the hormone hMG is an alternative to animal hyperovulation and estrus synchronization [19]. [21]. In animals, the use of hMG for hyperovulation increases follicle size and improves pregnancy. Higher rate and number of embryos and lower cost [11]. Therefore, research is needed to enable postmenopausal females to produce hMG1. Because FSHLH like was first manufactured in the form of an injectable hormone of 150 IU for human reproductive health. The most important ingredient that supports hMG is well known as the balanced FSHLH content, ie FSH 75 IU and LH 75 IU, or the composition of FSH: LH 50%: 50%. hMG can be used directly in the process of human in vitro maturation (IVM) and in vitro fertilization (IVF) for female infertility. Treatment with hMG results in very satisfactory egg collection and embryonic development [1- 11].

2. MATERIALS AND METHODS

After determining the level of crude hMG urine for menopausal women using the elisa method, it was also determined that the level of 10 IU crude hMG was injected intra peritoneally into female white rats., T₁ was injected with 10 IU of hMG Crude hMG urine of menopausal intra peritoneal dissolve in 30% Polyvinylpyrrolidone (PVP) [28- 34]. T₂ 10 white rats were injected with 10 IU hMG Crude hMG urine for intra peritoneal menopausal women dissolve propylene glycol (PG) in a ratio of 1:4 [35- 42]. The injection of 10 IU hMG hMG was carried out at the basic estrus synchronization. After 48 hours continued 10 IU hCG chorulon was injected and waited for 17 hours, then surgery was performed. observations were made of the injection site for intra peritoneal histological changes, in the kidneys, liver and ovaries in groups T₀, T₁ and T₂, respectively.

3. RESULTS AND DISCUSSION

Research Results 2 Observations were made at the intra peritoneal injection site in 30 white rats, with every ten males given 1 male where the former injection site was 10 IU hMG after 48 hours. After 17 hours of hCG chorulon 10 IU injection, the observation of histological changes in the peritoneum, kidney, liver and ovaries were divided into groups T₀ (10 Rats), T₁ (10 Rats) and T₂ (10 Rats) respectively. No histologic changes were found on the peritoneal surface as a result of injection of hMG 10 IU hMG (T₀) Crude hMG in Polyvinylpyrrolidone (PVP) T₁ or Crude hMG in soluble propylene glycol (PG) [41] T₂ where the real aim of administering PVP and PG solvents is to obtain a dose of single in order to obtain the results of gonadotropin activity for ease of use in experimental animals $P > 0.05$, meaning that even though 10 IU hMG was used it did not cause histological changes in the tissue studied. However, in white rat tissue, intraperitoneal injection of hMG 10 IU caused changes and growth of follicles on the surface of the ovary until ovulation occurred. Observations were made at the intra peritoneal injection site on 30 white rats, every 10 males were given 1 male where the former was injected with 10 IU hMG after 48 hours. After 17 hours of injection of hCG chorulon 10 IU, histological changes were observed in the peritoneum, kidney, liver and ovaries divided into groups T₀ (10 rats), T₁ (10 animals) and T₂ (10 animals) respectively. No histologic changes were found on the peritoneal surface as a result of injection of hMG 10 IU hMG (T₀) Crude hMG in Polyvinylpyrrolidone (PVP) T₁ or Crude hMG in soluble propylene glycol (PG) [41] T₂ where the real purpose of administering PVP and Solvent PG is to get a single dose so that the results of gonadotropin activity for ease of use in experimental animals $P > 0.05$ means that even though 10 IU hMG is used it does not cause

histological changes in the tissue studied. However, in white rat tissue, intraperitoneal injection of hMG 10 IU caused changes and growth of follicles on the surface of the ovary until ovulation occurred. At intervals of 1 week, 3 weeks or 9 weeks after the injection, 10 mice from each group were anaesthetized with ether and the tissue surrounding the injection site removed. The removed tissue was immediately fixed in 4% paraformaldehyde/0.05 M phosphate buffered solution. The samples were embedded in paraffin and sectioned specimens stained with haematoxylin and eosin (HE).

The histology of the stained sections was examined by light microscopy. Haematoxylin and eosin-stained sections of tissue surrounding the injection site as viewed by light microscopy. Polyethylene glycol was injected subcutaneously into the mouse 3 weeks earlier. Slight inflammatory cell infiltration can be observed ($\times 100$) (kawakami,2004). major renal histopathological changes during different stages of EG poisoning, which may be helpful when determining the date of EG poisoning itself. A single center retrospective study conducted on all cases of EG poisoning showed that in the early stages of EG poisoning, fine crystalline dust was deposited onto the basement membrane of tubular cells, followed by internalization of calcium oxalate crystals into epithelial cells. Then, the crystals form larger aggregates within the epithelial cells. As the changes progress, tubular epithelial damage occurs with repeated therapy [47], [48]. Tests were performed on 10 New Zealand white rabbits after PVP injection into the knee joint for 3, 7, 14 and 30 days and submitted for macroscopic and histological evaluation. The test results were compared with the data obtained after injection of normal saline. Macroscopically there was no change in the boundaries of the articular capsule and cartilage; there was little and no synovial membrane enlargement in the first 7 days after PVP injection. In histological tests it was observed that the reaction in the knee joint after PVP injection is characterized by a single inflammatory chain without the significant participation of neutrophils [50]. The efficacy of a single intramuscular dose of 450 or 600 international units (IU) of human menopausal gonadotropin (hMG) or 30 mg of follicle stimulating hormone (FSH), each dissolved in 30% polyvinylpyrrolidone K-30 (PVP), for superovulation treatment was compared to that of superovulation induction by administration of a total dose of 600 IU hMG given in declining doses twice daily over a 3-day period in black cattle no changes of side effect [51].

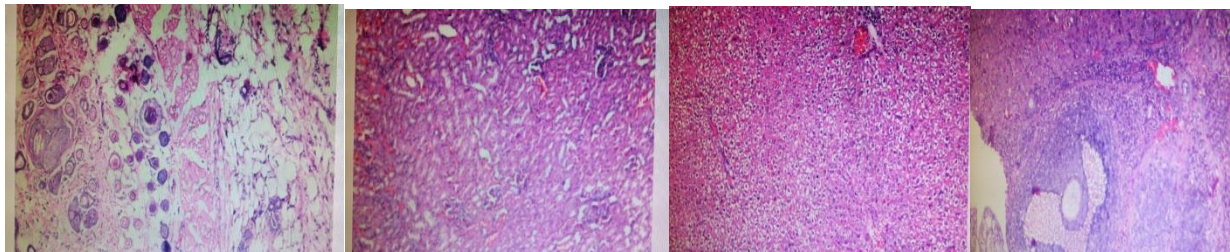


Fig 1.10 IU Crude hMG as control group without Polyvinylpyrrolidone (PVP) and propylene glycol (PG) There was no change in Peritoneum, Kidney and liver tissue after injection and the development of dominant follicles in the ovaries

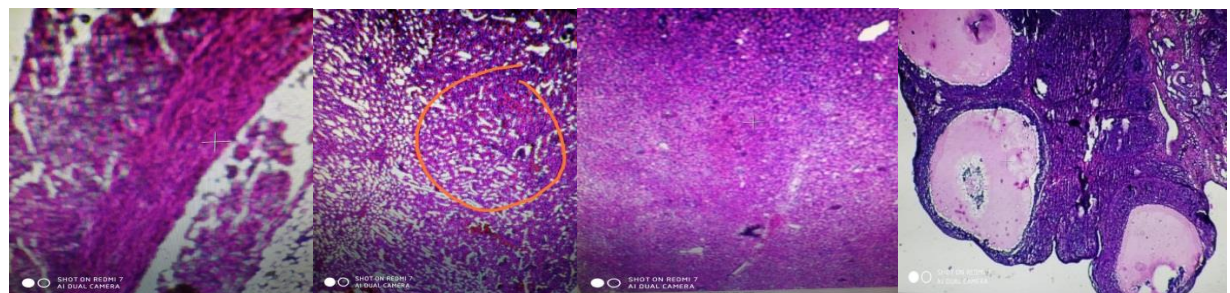


Fig 2.10 IU Crude hMG in Polyvinylpyrrolidone (PVP) There was no change in Peritoneum, Kidney and

liver tissue after injection and the development of dominant follicles in the ovaries

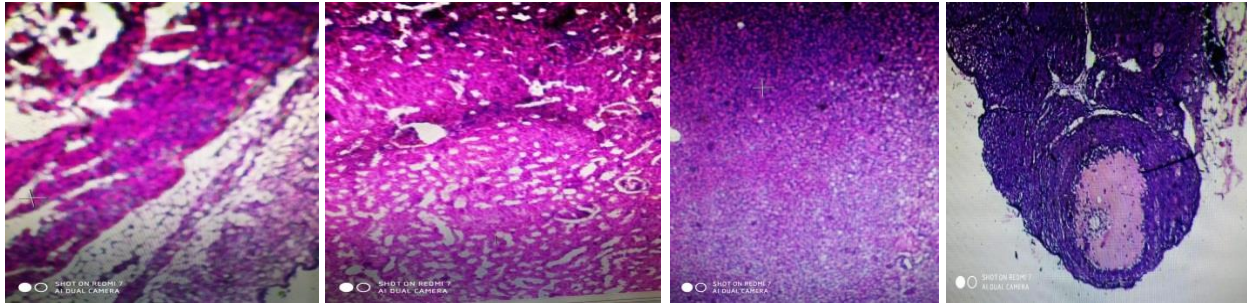


Fig 3. 10 IU Crude hMG in soluble propylene glycol (PG) There were no changes in Peritoneum, Kidney and liver tissue after injection and the development of dominant follicles in the ovaries

4. Conclusion

Polyvinylpyrrolidone (PVP) or dissolved in propylene glycol (PG) could not cause histological changes in the studied tissues. However, in rat tissue, intra-peritoneal injection of hMG 10 IU caused changes and growth of follicles on the surface of the ovary until ovulation occurred $P > 0.05$.

5. REFERENCES

- [1] Bruno Lunenfeld, Thomas D'Hooghe, ,*Wilma Bilger,Salvatore Longobardi,V.Alam, and Sesh K. Sunkara .2019.The Development of Gonadotropins for Clinical Use in the Treatment of Infertility..Front Endocrinol (Lausanne).10: 429.
- [2] European Medicines Agency GONAL-f. (2018). Available online at: <https://www.ema.europa.eu/en/medicines/human/EPAR/gonal-f> (accessed January 29, 2019).
- [3] European Medicines Agency Puregon. (2018). Available onlineat: <https://www.ema.europa.eu/en/medicines/human/EPAR/puregon> (accessed January 29, 2019).
- [4] Medicines Agency Rekovelle: Follitropin Delta. (2017). Available online at: <http://www.ema.europa.eu/ema/index.jsp?duct> was made as a Frozen Dry form.
- [5] J. W. Mcarthur, A. Howard, A. Somerville, R. Perley, And C. Keyes. 1967. Relative Recovery of Follicle-Stimulating Hormone and Luteinizing Hormone from Post-Menopausal Urine by the Albert and Johnsen . The Endocrine Society. p.530.
- [6] Manob Tangtrongpiros, Khamchai Lawonyawut and Sujin Nukwan.1988. Induced Spawning By Using hCG Produced From Urine of Pregnant Women. National Inland Fisheries Institute Bangkhen, Bangkok. Thailand.
- [7] Kongsak, D. 1984. Hormone Treatment and Diagnosis of Clinical Gynecologic Endocrinology, Faculty of Medicine, Ramathibhadhi Hospital, Bangkok, 153 p.
- [8] Van De Weijer, B. H.; Mulders, J. W.; Bos, E. S.; Verhaert, P. D.; Van Den Hooven, H. W. (2003). "Compositional analyses of a human menopausal gonadotrophin preparation extracted from urine (menotropin). Identification of some of its major impurities". *Reproductive Biomedicine Online*. 7 (5): 547–557. doi:10.1016/S1472-6483(10)62071-8. PMID 14680547.

- [9] Lunenfeld B (2004). "Historical perspectives in gonadotropin therapy". *Human Reproduction Update*. 10 (6): 453–467. doi:10.1093/humupd/dmh044. PMID 15388674.
- [10] H, Balen A, Jansen CA (October 2002). "Prion transmission in blood and urine: what are the implications for recombinant and urinary-derived gonadotrophins?". *Hum. Reprod.* 17 (10): 2501–8. doi:10.1093/humrep/17.10.2501. PMID 12351519.
- [11] Bagratee, J. S.; Lockwood, G.; López Bernal, A.; Barlow, D. H.; Ledger, W. L. (1998). "Comparison of highly purified FSH (metrodin-high purity) with pergonal for IVF superovulation". *Journal of Assisted Reproduction and Genetics*. 15 (2): 65–69. doi:10.1007/BF02766827. PMC 3455420. PMID 9513843.
- [12] Daya, S.; Ledger, W.; Auray, J. P.; Duru, G.; Silverberg, K.; Wikland, M.; Bouzayen, R.; Howles, C. M.; Beresniak, A. (2001). "Cost-effectiveness modelling of recombinant FSH versus urinary FSH in assisted reproduction techniques in the UK". *Human Reproduction (Oxford, England)*. 16 (12): 2563–2569. doi:10.1093/humrep/16.12.2563. PMID 11726575.
- [13] Van Wely, M.; Kwan, I.; Burt, A. L.; Thomas, J.; Vail, A.; Van Der Veen, F.; Al-Inany, H. G. (2011). Van Wely, Madelon (ed.). "Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles". *The Cochrane Database of Systematic Reviews* (2).
- [14] Practice Committee Of American Society For Reproductive Medicine, Birmingham (November 2008). "Gonadotropin preparations: past, present, and future perspectives". *Fertil. Steril.* 90 (5 Suppl): S13–20. doi:10.1016/j.fertnstert.2008.08.031. PMID 19007609
- [15] Mayer, L. and P. Hoyer. 2005. A New Hormonally Relevant Model for human Menopause. *Jax Mice Literatur*. No. 496.
- [16] IBM SPSS . 2012. Statistics Base window.
- [17] Rudy AN, 2018. Recognize mice as laboratory animals. *Mulawarman University Perss*. 146 -147
- [18] Laurence A. Cole, M.D., Ph.D. Yasushi Sasaki, M.D., Ph.D. Carolyn Y. Muller, M.D. 2007. Normal Production of Human Chorionic Gonadotropin in Menopause. *The New England Journal of Medicine* 356:11.
- [19] R Bassett , D Ceccarelli, C Crisci, A M di Tria, M Mancinelli, F Martelli, D Mendola, A Pezzotti. 2005. Comparative analytical characterization of two commercial 1 human folliclestimulating hormones extracted from human urine. *Curr Med Res Opin* 21(6):899-905.
- [20] Levi Setti PE, Alviggi C, Colombo GL, Pisanelli C, Ripellino C, Longobardi S, 2015 et al. Human recombinant follicle stimulating hormone (rFSH) compared to urinary human menopausal gonadotropin (HMG) for ovarian stimulation in assisted reproduction: a literature review and cost evaluation. *J Endocrinol Invest*. (2015) 38:497–503.
- [21] Bruno Lunenfeld , 2004. Historical perspectives in gonadotrophin therapy. Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel. *Human Reproduction Update*, Vol.10, No.6 pp. 453–467
- [22] Grkovic, I. Immunohistochemistry. Department of Anatomy, Histology and Embryology, University of

Split. 2007

[23] 2014. IHC and ISH advanced staining. Leica Biosystems.

[24] Stanford University. 2013. Immunohistochemical stains.

[25] Kiupel, M. 2010. Diagnostic molecular pathology. Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University

[26] Peckham, A. 2003. Immunohistochemistry. Faculty of Biological Sciences, Leeds University,

[27] CCPL. 2015. Special stains and immunohistochemistry. Central Coast Pathology Laboratory.

[28] Yamamoto M, Ooe M, Kawaguchi M, Suzuki T 1994. Superovulation in the cow with a single intramuscular injection of FSH dissolved in polyvinylpyrrolidone. *Theriogenology*;41:747–55.

[29] T, Aoyagi Y, Konishi M, Kishi H, Taya K, Watanabe G, et al. 1995 Superovulation of Holstein heifers by a single subcutaneous injection of FSH dissolved in polyvinylpyrrolidone. *Theriogenology*;43:1259–68.

[30] Satoh H, Numabe T, Takada T, Oikawa T, Kifune A, Watanabe G, et al. 1996 Superovulation in Japanese beef cows using polyvinylpyrrolidone (PVP) as the vehicle for porcine FSH (pFSH). *Theriogenology*;45:332 (Abstract).

[31] Takedomi T, Aoyagi Y, Konishi M, Kishi H, Taya K, Watanabe G, et al. 1993 Superovulation in Holstein heifers by a single injection of porcine FSH dissolved in polyvinylpyrrolidone. *Theriogenology*;39:327 (Abstract).

[32] Suzuki T, Yamamoto M, Ooe M, Takagi M. 1994. Superovulation of beef cows and heifers with a single injection of FSH diluted in polyvinylpyrrolidone. *Vet Rec*;135:41–2.

[33] Yamamoto M, Suzuki T, Ooe M. 1993 Superovulation in Beef Cows and Heifers with a Single Injection of FSH dissolved in Polyvinylpyrrolidone. *J Reprod Dev*; 39: 353-356.

[34] Yamamoto M, Suzuki T, Ooe M, Takagi M, Kawaguchi M 1992. Efficacy of single vs multiple injection superovulation regimens of FSH using Polyvinylpyrrolidone. *Theriogenology*; 37: Abstract 325.

[35] Ielsen, and Nicolaj .2004. "Propylene glycol for dairy cows". *Animal Feed Science and Technology*. 115 (3–4): 191–213. doi:10.1016/j.anifeedsci.2004.03.008

[36] "PROPYLENE GLYCOL - CAMEO. 2018. Chemicals". NOAA Office of Response and Restoration. NOAA.

[37] "Propylene glycol". 2020. pubchem.ncbi.nlm.nih.gov. Retrieved 27 April 2020. This article incorporates text from this source, which is in the public domain.

[38] ^ "Propylene glycol and cats. 2013" (PDF). Archived from the original (PDF) on 201502-27.

- [39] Wikipedia.2020. Propylene glycol the free encyclopedia.
- [40] Zurisaday Santos-Jimenez 1,2, Sara Guillen-Gargallo 3 , Teresa Encinas 1 , Fiammetta Berlinguer 4 , Francisco G. Veliz-Deras 2 , Paula Martinez-Ros 3,* and Antonio GonzalezBulnes .2020.Use of Propylene-Glycol as a Cosolvent for GnRH in Synchronization of Estrus and Ovulation in Sheep. *Animals* 2020, 10,897 7.
- [41] Lopez-Sebastian, A.; Gomez-Brunet, A.; Lishman, A.W.; Johnson, S.K.; Inskip, E.K.1993. Modification by propylene glycol of ovulation rate in ewes in response to a single injection of FSH. *J. Reprod. Fertil.* 1993, 99, 437–442.
- [42] Propylene glycol".2020 pubchem.ncbi.nlm.nih.gov (in English). Retrieved April 27, 2020. This article incorporates text from that source, which is in the public domain.
- 43.Rui Hua, Lan Ma, and Hong Li.2013. Clinical Effects of a Natural Extract of Urinary Human Menopausal Gonadotrophin in Normogonadotropic Infertile Patients. *International Journal of Reproductive Medicine.* 1-4.
- [44] Risquez F 2001 Induction of follicular growth and ovulation with urinary and recombinant gonadotrophins. *Reproductive BioMedicine Online* 3, 54–72.
- [45] Sandro C. Esteves.2015.Efficacy, efficiency and effectiveness of gonadotropin therapy for infertility treatment *MedicalExpress* (São Paulo, online)
- [46] Leão RdeB, Esteves SC. 2014, Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech. *Clinics (Sao Paulo).*;69(4):279-93.
- [47] T kawakami, T Mizoguchi , M Ito , , S Matsuura, , T Shimizu, , S Kurihara And T Kawai .2004 Histopathological Safety Evaluation of Polyethylene Glycol Applied Subcutaneously in Mice T, *The Journal of International Medical Research* 2004; 32: 66 – 69
- [48] Janos Bokor, Krisztina Danics, Eva Keller and Zoltan Szollosi.2018. Time-dependent changes in kidney histopathology in ethylene glycol poisoning *Medicine, Science and the Law* 0(0)
- [49] McMartin K. 2009. Calcium oxalate crystals in acute ethylene glycol poisoning: a confocal laser scanning microscope study in a fatal case. *Clin Toxicol (Phila).* . 47(9):859-69
- [50] Stanisław Pielka 1, Bogusława Zywicka, Janusz Rosiak, Artur Henke, Piotr Ulański, Danuta Paluch, Maria Szymonowicz, Leszek Solski, Jolanta Staniszevska-Kuś. 2004. Tissue reaction after injection of polyvinylpyrrolidone (PVP) preparation into knee joint. *Experiment] Polim Med.* 34(4):3-8
- [51] Sugano, M. and Shinogi, T. (1999) Superovulation induction in Japanese Black cattle by a single intramuscular injection of hMG or FSH dissolved in polyvinylpyrrolidone, *Anim. Reprod Sci.* 55, 175–181.