

## IMPACT OF SULPIRIDE AND PMSG HORMONE TREATMENT ON ESTRUS AND NUMBER OF RESULTED OFFSPRINGS IN FEMALE RATS

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### ABSTRACT

This study aimed to evaluate the impact of sulpiride and PMSG hormone treatments on estrus and the number of offspring produced in female rats. Thirty mature rats were divided into three groups, each consisting of 10 females. The first group was treated intraperitoneally with sulpiride (0.25 mg/kg), the second group with PMSG hormone (20 IU/kg), and the third group with normal saline. Treatments were administered after the onset of estrus. Following treatment, natural mating occurred. The results of the study showed no significant differences between the PMSG hormone, sulpiride, and normal saline treatment groups regarding the number of females that showed estrus and mated. However, a significant difference ( $P < 0.05$ ) was observed between the PMSG treatment group and the other groups with respect to the average number of embryos, which were 128, 75, and 55, respectively. The Luteinizing Hormone (LH) concentration was significantly higher ( $P < 0.05$ ) in the sulpiride-treated group ( $1.44 \pm 0.12$  ng/ml) compared to the PMSG hormone ( $1.23 \pm 0.14$  ng/ml) and normal saline treatment groups ( $0.76 \pm 0.08$  ng/ml). In conclusion, the application of PMSG was more effective in inducing ovulation and increasing the average number of offspring compared to sulpiride treatment in female rats.

**Keywords:** Sulpiride, PMSG, Ovulation, offspring, Rat.

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### INTRODUCTION

Experimental animals play a crucial role in teaching and research. Small rodents like mice and rats are more preferred to larger animals like rabbits, dogs and cats (Amin *et al.*, 1996). In reproductive function research, particularly those involving the use of female animals, mice, and rats are commonly used, possibly due to their well-characterized estrous cycle and secure handling. The short and precise length of estrous in these rodents (Andrews, 1981, Auta, 2016) also makes

them very suitable. It can also be used to investigate the effects of drugs and chemicals on reproductive function (Byers, 2012; Younis *et al.*, 2019). It is important to obtain a large number of ova for some developmental biotechnology techniques, such as for the generation of transgenic and cloned animals. In veterinary medicine, synchronization is important using certain medication. Administration of pregnant mare serum gonadotropin (PMSG) is a simple method to induce superovulation, and this method is widely used in mice and rats. In the mouse, superovulation can be induced at any stage of the estrous cycle by the injection of PMSG (Poernomo, 2013, Bambang, 2016). The maternal exposure to sulpiride (sulpiride is a substituted benzamide derivative and act

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as a selective dopamine D2 antagonist indicated to treat acute and chronic schizophrenia) can alter reproductive function in female offspring rats (De Azevedo Camin *et al.*, 2015; Younis *et al.*, 2020). Sulpiride significantly reduced freezing expression in female rats and the females' responsiveness to sulpiride appears to be dependent on the estrous cycle phase. A significant reduction in the expression of contextual conditioned freezing behavior after sulpiride administration was observed in females in proestrus/estrus, but not in metestrus/diestrus (Reimer *et al.*, 2018). Sulpiride has been successfully used for induction of ovulation in ewes (Naqvi *et al.*, 2017). There is an increase in LH secretion induced by the sulpiride in animals exposed to the inhibitory role of dopaminergic pathways (Fiorica *et al.*, 2015; Giorgi *et al.*, 2015; Saxena *et al.*, 2014). Administrations of dopamine antagonists such as sulpiride in mares resulted in a hastening of first ovulations without interference with fertility (Panzani *et al.*, 2011). In the fertility study, sulpiride-treated females, showing persistent diestrus, resulted in successful mating and almost all females got pregnant (Ishii *et al.*, 2009).

This study aimed to evaluate the impact of sulpiride and PMSG hormone treatment on estrus and the number of resulting offspring in female rats.

## MATERIALS AND METHODS

The study was conducted on 30 adult female rats, with average body weight ranging from 220 to 250g, during the (January 2023 to March 2023). The experiment was carried out at Tikrit University's College of Veterinary Medicine in the animal house. The animals divided to three groups each one containing 10 females. The number of animals in each cage was two and was

monitored during the test stages for nutrition and other environmental factors. The first group was treated with sulpiride (Solarbio Life Science, China), a dose of 0.25 mg/kg injected intraperitoneally, second group was treated with PMSG hormone (MSD Animal Health, Folligon® PMSG, USA) 20 IU/kg injected intraperitoneally and a third group treated by normal saline 0.2 ml injected intraperitoneally. After treated the males were introduced to the females for natural mating.

### Blood collection

Blood samples were collected on the same days after 10 hours from treatment to determine serum Luteinizing Hormone (LH) concentrations in all groups. The blood collection from the tail vein was 0.5 ml while the rats were under anesthesia (Van Herck *et al.*, 2001).

### Hormonal assay

LH was assayed by using kits of Enzyme-Linked Immunosorbent Assay (ELISA) Kits specific for the estimation of rat Luteinizing hormone (LH) (ELK Biotechnology) (China).

### Statistical Analysis

Data were analyzed by Minitab program system version 17 and ANOVA tests were applied. The means compared by Duncun's multiple ranges under the level of significant  $P \leq 0.05$ .

## RESULTS

The result presented in table (1) showed that there were non-significant differences in females showing estrous between all groups. The results showed a significantly higher number of offspring ( $P < 0.05$ ) in the group treated with PMSG in comparison with other groups.

**Table 1:** Effects of PMSG, sulpiride treatments on estrus expression and average number of offspring in female rats.

Groups	treatment	No. of Mated	No. of offspring	Average of offspring
G1 (10 female)	Sulpiride	10	75	7.5 b
G2 (10 female)	PMSG	10	128	12.8 a
G3 (10 female)	N. saline	10	55	5.5 c

$P < 0.05$ , a significant difference.

The luteinizing hormone concentration in the sulpiride-treated group was significantly ( $P < 0.05$ ) higher than that recorded in the PMSG and N. Saline group ( $1.44 \pm 0.12$ ,  $76 \pm 0.08$  ng/ml, respectively).

**Table 2:** LH hormone concentration in female rats after treatment.

Groups	treatment	LH (ng/ml)
G1 (10 female)	Sulpiride	$1.44 \pm 0.12a$
G2 (10 female)	PMSG	$1.23 \pm 0.14b$
G3 (10 female)	N. saline	$0.76 \pm 0.08c$

$P < 0.05$ , different superscripts mean significantly difference.

## DISCUSSIONS

It was documented from the current work that the average number of offspring increased in female rats after the application of PMSG. This result agrees with previous findings by Poernomo (2013), who reported an increase in the number of ova and embryos with the use of PMSG. This study also aligns with other authors who reported an increase in the number of embryos (Mukumoto *et al.*, 1995; Nooranizadeh *et al.*, 2018). Bó and Mapletoft (2014) reported that PMSG is used for the induction of superovulation in rats, and this result agrees with the present study due to the increased number of offspring.

The current study found that sulpiride treatment had non-significant effects on the number of produced offspring, which is consistent with the results recorded by Ishii *et al.* (2009). They reported that sulpiride-treated females showed persistent diestrus, resulting in successful mating, with almost all females becoming pregnant, although implantation loss increased under the effect of sulpiride. The same authors attributed

this to the negative effects of sulpiride on oocyte development. Our results also coincide with those of other authors, where sulpiride treatment showed clear effects on the estrus stage in female rats (Hannigan *et al.*, 1997; Mostafapour *et al.*, 2014). However, these results disagree with other studies that reported sulpiride's use to induce ovulation (Ali and Najlaa, 2023; Al-Mousawe and Ibrahim, 2024).

This study reported that LH levels in the sulpiride treatment group were significantly higher than in other groups, which agrees with findings reported by OTA *et al.* (1986). However, this finding disagrees with those reported by Ahmadi *et al.* (2013) and Mostafapour *et al.* (2014). Additionally, this study disagrees with the notion that sulpiride treatment reduces the synthesis and secretion of GnRH in the hypothalamus, which would decrease stimulation for LH and FSH secretion in the pituitary gland (Wieck and Haddad, 2003).

## CONCLUSION

It was concluded that the application of PMSG had the best effects on estrus manifestation and the average number of produced offspring compared with sulpiride treatment in female rats.

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The authors declare that the present study has no financial issues to disclose.

**Conflict of interest**

The author declare that there is no conflict interest.

**Author's contributions**

Ali A. Abd: Research article, funding the acquisition and preparing materials and review, explain the finding, experimental design, statistical analysis and editing.

**Ethical approval**

It was granted through the local committee of the animal care and use at the College of Veterinary Medicine, University of Tikrit (No. Tu. Vet. 25/2024).

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## تأثير العلاج بهرمون مصل الفرس الحامل والسليبرايد على الشبق وعدد المواليد في إناث الجرذان

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هدفت هذه الدراسة إلى تقييم تأثير العلاج بهرمون PMSG والسليبرايد على الشبق وعدد المواليد في إناث الجرذان. تم تقسيم ثلاثين أنثى ناضجة إلى ثلاث مجاميع تحتوي كل منها على ١٠ إناث. المجموعة الأولى تم معالجتها بالسليبرايد (٢٥, ٠ مجم / كجم) محقوناً داخل البريتون، والمجموعة الثانية تم معالجتها بهرمون PMSG (٢٠ وحدة دولية / كجم) محقوناً داخل البريتون والمجموعة الثالثة تم معالجتها بمحلول ملحي طبيعي محقوناً داخل البريتون. تم علاج جميع المجاميع بعد بدء الشبق. وتم تلقيح إناث الجرذان بشكل طبيعي بعد العلاج. أظهرت نتيجة هذه الدراسة عدم وجود فرق معنوي بين مجاميع العلاج بهرمون PMSG والسليبرايد ومحلول الملحي الطبيعي وفقاً للإناث التي أظهرت الشبق والتزاوج، بينما لوحظ وجود فرق معنوي  $P < 0.05$  بين علاج PMSG والمجموعات الأخرى فيما يتعلق بمتوسط الأجنة ١٢٨ و ٧٥ و ٥٥ على التوالي. كان تركيز هرمون LH أعلى بشكل ملحوظ ( $P < 0.05$ ) في المجموعة المعالجة بالسليبرايد ( $١,٤٤ \pm ٠,١٢$  نانوغرام/مل) بالمقارنة مع هرمون PMSG ( $١,٢٣ \pm ٠,١٤$  نانوغرام/مل) والعلاج بالمحلول الملحي الطبيعي ( $٠,٧٦ \pm ٠,٠٨$  نانوغرام/مل). وفي الختام نستنتج ان العلاج بهرمون PMSG كان فعالاً في تحفيز التبويض وزيادة معدل المواليد بالمقارنة مع العلاج بالسليبرايد في إناث الجرذان.

**الكلمات المفتاحية:** سوليبرايد، مصل الفرس الحامل ، التبويض، النسل، الفئران .