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EFFECT OF REPEATED SUPEROVULATION TREATMENTS ON EMBRYO YIELD AND CHROMOSOMAL ABERRATIONS IN MICE

H.M.El Ashmaoui,[1] A.H.Barkawi [2] and I.A.H.Barakat[1]

1- Cell Biology Departement, National Research Center, Dokki, Giza, Egypt, 2-Departement of Animal Production, Faculty of Agriculture, Cairo,University, Giza, Egypt

SUMMARY

The effect of repeated gonadotrophic hormonal injection to induce superovulation on embryo survivability and chromosomal aberrations in mice were studied . Thirty adult female mice were divided into two equal groups and injected for three times with two differents regimes (2.5I.U.FSH +5 I.U.HCG and 5 I.U. PMSG +5 I.U. HCG). Five females from each group were sacrificed after each superovulatory treatment. No abnormal embryos were obtained when females were treated once with PMSG + HCG. Percentage of abnormal embryos increased when females were multi-treated for superovulation. Within each group significant difference was observed concerning the implantation sites and living embryos .However , there was difference in weight of embryos. Chromosomal gap was found in embryo cells obtained from donors treated twice and thrice for superovulation by PMSG or FSH. Polyploidy aberration was found in embryo cells obtained after three times hormonal treatment. Chromosomal aberrations after (FSH + HCG previously) treatment were lower than in (PMSG + HCG) at any dose of treatment.

The obtained results indicated that the FSH + HCG treatment was the appropriate hormonal preparation for superovulating mice, as it resulted in low chromosomal aberrations, more implantation sites and change in no.of living embryos.

Keywords: *Supperovulation,embryo,chromosomal aberrations, mice*

INTRODUCTION

Spreading the genetic materials of superior females depends on the regular program of superovulation to maximize the number of the produced embryos. The response to superovulation and gained embryos decrease significantly with the repetition of hormonal manipulation. This phenomena was reported on ewe (Sharma *et al.*, 1996) and in cattle (Totey *et al.*, 1992). Moreover, formation of abnormal embryos increases with superovulatory treatments (King and Picard, 1985).

Many reports attributed the low response of donors after repeated treatment to either antibody formation against the injected hormones (Lin and Bailey, 1965 and Totey *et al.*, 1992) or incidence of chromosomal aberrations, which are recognized as one of the primary causes of reproductive failure and early embryonic mortality in

domestic animals (Boue and Boue, 1973 and King, 1985), however, the mode of action of superovulation treatments on chromosomal abnormalities is not completely determined yet.

The present study was planned to test the response of repeated hormonal treatment for induction of superovulation and chromosomal changes leading to low number and less quality of mice embryos.

MATERIALS AND METHODS

Thirty healthy mature female and ten male mice weighting 20 to 30 g each of Swiss Albino Strain were obtained from the animal house of the National Research Center. Animals were housed separately in appropriate cages measured 35 X 25 X 25 cm. each. Animals were provided with food and water *ad-libitum*.

Females animals were divided into two equal groups according to the type of treatment for superovulation. Each group was subdivided into three equal classes according to the number of superovulation treatments (once, twice or thrice).

Females of the first group (G1) (n=15) was superovulated using FSH + HCG. Hormons dose were injected subcutaneously (S.C.) during the diestrous phase (2 days before the expected day of estrous).For a single superovulating treatment females were injected with a dose of 2.5 I.U. of FSH and HCG 2x daily at a time interval of 12hr starting at 7 a.m for 5 successive days with FSH (from Metodrine, Sereno, S.P.A.).After the final injection, females were injected intraperitoneally (i.p) with 5.0 I.U. of HCG (Pregnyl, produced by the Nile Co., Egypt, under licence of N.V. Organon OSS Holand) at 15:00 hr. (Munoz *et al.*, 1994). Five females were chosen randomly to be mated with the males, they were killed at day 13 post mating (G1-1). After 7 days from the beginning of the first treatment the rest of females (n = 10) were re injected for the second time following the same procedure. Five females were chosen randomly and allowed to be mated with males before killing at day 13 from pregnancy (G1-2). Seven days after, the living five females were treated similarly for the third time and were killed 13 days post mating (G1-3).

Females of the second group (G2) (n = 15) were superovulated using PMSG and HCG. Each female was injected i.p. with 5.0 I.U. of PMSG (Sigma Chemical Co., Gestyl, U.S.A). Approximately, 48 h. later females were injected i.p. with 5.0 I.U. of HCG (Henery and Kaufman, 1993) (G2-1). Seven days from the beginningof the first treatment, the rest females were re-treated as the procedure described in the G1, the females of all classes were killed at day 13 post mating.

At day 13 of pregnancy after killing the donors, five embryos were collected randomly from the uterus to study the morphological changes and the chromosomal aberration types according to Evans (1987). For each embryo, 50 cells in metaphase were examined to check the chromosomal aberrations.

Statistical analysis

Data of this study was subjected to 2 X 2 factorial design and the analysis was conducted applying the General Linear Model (SAS, 1990).

RESULTS AND DISCUSSION

1- Response to superovulation

Repeated treatment for superovulation with PMSG showed a positive relationship between the times of treatment and ovulation response as indicated by the number of implantation sites, while in the FSH treated group there is no effect (Table 1). Concerning the number of living embryos, no difference between the two different hormonal treatment, except in the second injection.Ithe same treatments, no difference between G2-1 and G2-2 , there was difference between G2-1 and G2-3,While in the G1, there was difference between G1-1 and G1-2.

Table 1. Embryo and pregnancy characteristics ($\overline{X}$±S.E) for female mice repeat treated with 2.5 I.U. FSH+5 I.U. HCG (G1) and 5 I.U. PMSG+5 I.U. HCG (G2)

Group	Trait	Implantation Sites (No.)	Living Embryos (No.)	Abnormal embryos (retarded&absorbed) (No.)	weight (gm)
G1-1	$\overline{X}$ ±S.E	24.2± 3.4 ^{abc}	9.8± 3.2^{a}	14.2± 0.4^{b}	0.1± 0.01e
	Total no.	120	49	71	79
G1-2	$\overline{X}$± S.E	29.6± 1.0^{a}	18.6±0.8^{b}	17.8± 1.5^{ab}	0.1± 0.0^{d}
	Total no.	182	93	89	94
G1-3	$\overline{X}$ ± S.E	28.2 ± 4.0^{ab}	13.6±3.9^{ab}	21.8±3.4^{a}	0.1± 0.0f
	Total no.	177	68	109	68
G2-1	$\overline{X}$ ± S.E	10.8 ± 3.4^{d}	10.8±3.4^{a}	0.0^{c}	0.16± 0.01^{a}
	Total no.	54	54	0	55
G2-2	$\overline{X}$ ± S.E	16.4± 4.3^{c}	12.8±2.6^{a}	4.0±2.0^{c}	0.1± 0.0^{b}
	Total no.	84	64	20	71
G2-3	$\overline{X}$ ± S.E	18.8± 2.6^{bc}	16.6± 2.7^{b}	3.6±0.7^{c}	0.1± 0.0^{c}
	Total no.	101	83	18	83

- a, b, c, d, e, f means with different superscripts within each treatment and between the two treatments in the corresponding category of repeated injection are significantly different (P<0.05).
- Fifteen mice embryos were used in each treatment group and data of each class was extracted from 250 examined field / group.

G1	G2
G1-1: treated once	G2-1: treated once
G1-2: treated twice	G2-2: treated twice
G1-3: treated thrice	G2-3: treated thrice

The number of abnormal embryos was higher ($P < 0.05$) in FSH than PMSG treatment. Also, it was less in female that treated once than those treated twice or thrice. Repeated injection with gonadotrophic hormones resulted in as increase of abnormal embryos (Table1) .

2- Chromosomal aberrations of embryos

Regarding the effect of repeated administration of hormonal preparation types on embryo cells, the results in Table (2) shows that a lower ($P<0.05$) incidence of either structural or numerical aberrations in embryo cells derived from female mice treated with FSH than those treated with PMSG. Additionally, the total aberrations increased with the frequency of hormonal treatment. The values of total aberrations in PMSG treated groups (C2-1, C2-2 and C2-3) were always higher than the values obtained in the corresponding group of FSH treatment (Table2).

Table 2. Chromosomal aberrations ($\overline{X}$±S.E) of embryos derived from female mice repeat treated with 2.5 I.U. FSH + 5 I.U. HCG (G1) and 5 I.U. PMSG + 5 I.U. HCG (G2)

Group	Trait	Structural	Numerical	Overal mean	Aberrant metaphase	CWA
G1-1	X± S.E	4.5± 0.2e	3.3 ± 0.2^{c}	7.8 ±0.2e	7.4 ± 0.2e	0.4 ± 0.1^{d}
	Total no.	111	83	194	184	10
G1-2	X ± S.E	7.2 ± 0.2^{d}	3.8 ± 0.2^{c}	11.0 ± 0.3^{d}	10.2 ± 0.2^{d}	0.8 ± 0.2^{bcd}
	Total no.	180	95	275	255	20
G1-3	X ± S.E	9.4 ± 0.3^{c}	4.8 ± 0.2^{b}	14.2 ± 0.3^{c}	12.9 ± 0.3^{d}	1.2 ± 0.2^{ab}
	Total no.	235	121	356	325	31
G2-1	X± S.E	7.6 ± 0.2^{d}	4.0 ± 0.2^{c}	11.6 ± 0.3^{d}	10.8 ± 0.3^{d}	0.7 ± 0.1^{cd}
	Total no.	190	99	289	270	18
G2-2	X ± S.E	10.8 ± 0.4^{b}	4.9 ± 0.3^{b}	15.7 ± 0.7^{b}	14.5 ± 0.6^{b}	1.2 ± 0.2^{abc}
	Total no.	271	122	393	363	30
G2-3	X ± S.E	12.5 ± 0.3^{a}	6.0 ± 0.2^{a}	18.4 ± 0.4^{a}	16.8 ± 0.4^{a}	1.6 ± 0.2^{a}
	Total no.	316	150	466	426	40

- a, b, c, d, e means with different superscripts within each treatment and between the two treatments in the corresponding category of repeated injection are significantly different ($P<0.05$).
- Fifteen mice embryos were used in each treatment group and data of each class was extracted from 250 examined field.
- C.W.A. Cells with more than one aberrant type

G1	G2
G1-1: treated once	G2-1: treated once
G1-2: treated twice	G2-2: treated twice
G1-3: treated thrice	G2-3: treated thrice

Table 3. Structural and numerical aberrations ($\overline{X}$ ± S.E) of embryos derived from female mice repeat treated with 2.5 I.U. FSH + 5 I.U. HCG (G1) and 5 I.U. PMSG + 5 I.U. HCG (G2)

Group	Trait	Structural				Numerical	
		Gap	Chromatid break	Centromeric attenuation	Chromosomal gap	Peridiploidy	Polyploidy
G1-1	$\overline{X}$± S.E	1.0± 0.2^{d}	1.2±0.2e	2.2± 0.2^{c}	0.0^{c}	3.3± 0.2^{d}	0.0^{c}
	Total no.	25	31	55	0	83	0
G1-2	$\overline{X}$± S.E	1.6± 0.2^{c}	3.2±0.1^{d}	2.4±0.1^{c}	0.0^{c}	3.8±0.2^{cd}	0.0^{c}
	Total no.	40	80	60	0	95	0
G1-3	$\overline{X}$ ± S.E	2.2 ± 0.2^{b}	3.8 ± 0.2^{c}	3.0±0.2^{b}	^{b}	4.4± 0.2^{bc}	0.4± 0.1^{b}
	Total no.	55	95	75	10	110	11
G2-1	$\overline{X}$ ± S.E	1.9 ±0.2^{bc}	3.3 ± 0.2^{d}	2.4± 0.2^{c}	0.0^{c}	4.0±0.2^{cd}	0.0^{c}
	Total no.	48	82	60	0	99	0
G2-2	$\overline{X}$ ± S.E	2.7 ± 0.1^{a}	4.5 ± 0.3^{b}	3.2±0.2^{ab}	0.4 ^{b}	4.9± 0.3^{ab}	0.0^{c}
	Total no.	68	112	80	11	122	0
G2-3	$\overline{X}$ ± S.E	3.0 ± 0.2^{a}	5.0 ± 0.2^{a}	3.6± 0.2^{a}	1.0 ^{a}	5.2±0.2^{a}	0.8±0.2^{a}
	Total no.	76	125	91	24	130	20

- a, b, c, d, e means with different superscripts within each treatment and between the two treatments in the corresponding category of repeated injection are significantly different (P<0.05).
- Fifteen mice embryos were used in each treatment group and data of each class was extracted from 250 examined field.
- G1 G2

G1-1: treated once G2-1: treated once
G1-2: treated twice G2-2: treated twice
G1-3: treated thrice G2-3: treated thrice

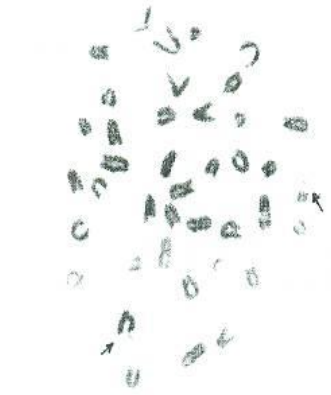

Fig. (1): A metaphase spread showing Break, and a Chromosomal gap

Fig. (2): A metaphase showing a Centromeric attenuation

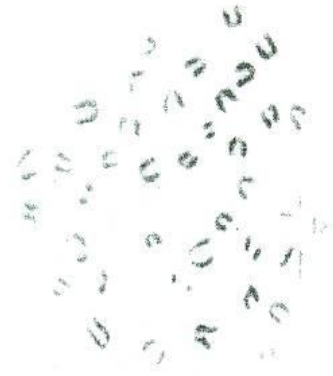

Fig. (3): A metaphase spread showing a monosomic cell (2n-1)

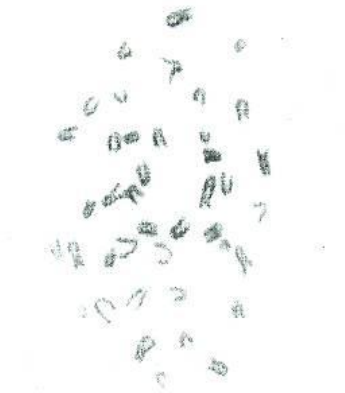

Fig. (4): A metaphase spread showing a trisomic cell (2n+1)

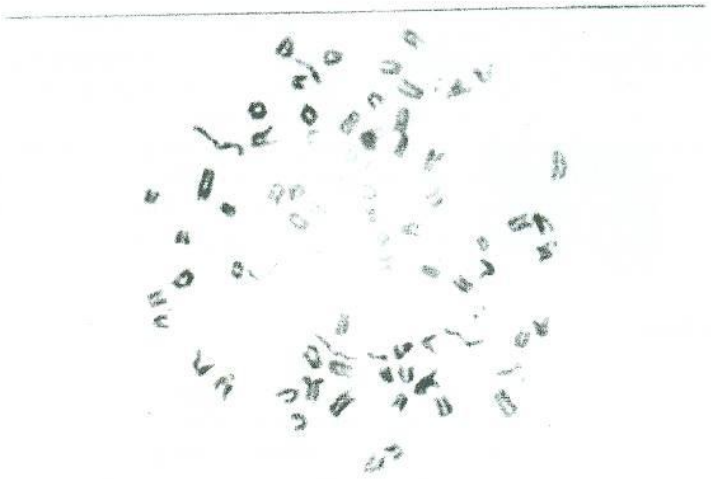

Fig. (5): A metaphase showing a polyploidy cell.

Structural and numerical types of chromosomal aberrations increased ($P<0.05$) after the 2nd injection for hormonal treatment in both hormones (FSH and PMSG) (Table 3). Gap, chromatid break (plate 1), and centromeric attenuation (plate 2) were the more frequent types of structural aberrations. Also, peridiploidy (plates 3 and 4) was the most frequent numerical aberration (Table 3). On the other hand, polyploidy was the lowest frequent numerical aberration (Table 3 and plate 5).

GENERAL DISCUSSION

So far, no data concerning the effect of repeated injection of gonadotrophic hormones on chromosomal aberrations are available. It seems that superovulation was associated with chromosomal aberrations when high doses of hormones were used. Soliman (1994) and Ma *et al.* (1997) found that there was a positive relationship between the PMSG dose and the incidence of polyploidy, as tested in zygote and intact embryos as well.

On the contrary, Yamamoto and Ingalls (1972); Maudlin and Fraser (1977) and Sengoku and Dukelow (1988) reported that the incidence of polyploidy was relatively low and there was no significant difference between treated and non-treated mice. Additionally, polyploidy was very low in both superovulated and non-superovulated female mice (Luckett and Mukherjee, 1986) and there was no

significant difference between treated mice groups, which disagrees with the finding of Girgis (1993). The difference is most probably due to the genotype, age of the female or the hormonal type.

Analysis of data, generally, revealed that all types of chromosomal aberrations were in the lowest value when females treated once for superovulation. With the repeated treatment, such aberrations increased gradually to reach the maximum after the 3rd treatment, particularly for those treated with PMSG.

In conclusion, FSH + HCG treatment appeared to be the best treatment for super-ovulating mice. It gave low chromosomal aberrations, high implantation sites and no detectable adverse effect on viability of embryos. Therefore, it would be recommended for inducing superovulation in mice. Also, it may be applied to large farm animals with possible expected particular results.

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تأثـــير إعـــادة معاملة التبويض المتعدد على إنتاج الأجنة والإختلالات الكروموسومية فى الفئران الصغيرة البيضاء

حسن ممدوح العشماوى[1]، أشرف هشام برقاوى[2]، إبراهيم حسن بركات[1]

١-قسم بيولوجيا الخلية، المركز القومى للبحوث، الدقى، الجيزة، مصر ٢- قسم الإنتاج الحيوانى، كلية الزراعة، الجيزة، مصر

أجريـــت هـــذه الدراســـة بهـــدف دراسة تأثير تكرار حقن إناث الفئران الصغيرة البيضاء بالهرمونات الجونادوتروفينـــية أو شبيهتها على عدد ووزن الأجنة الحية والغير طبيعية (الممتصة ومعاقة النمو) وكذلك عـــدد مواقـــع الإنغـــراس الجنينى بالإضافة إلى فحص كروموسومات خلايا الأجنة الناتجة من هذه الأمهات المعاملة هرمونياً من حيث التغيرات العددية والتركيبية.

اســـتخدم فـــى هذه الدراسة ثلاثون أنثى قسمت إلى مجموعتين متساويتين فى العدد. المجموعة الأولى حقنـــت بالهـــرمون المنشط للنمو الحويصلى (FSH) ثلاث مرات متتالية لبعضها بالإضافة لهرمون مشيمة الســـيدات الحوامل (HCG) بمعدل ٥ وحدات دولية لكل هرمون لعدد سبعة أيام وبعد كل معاملة يتم إختيار خمسة إناث عشوائياً للذبح فى اليوم الثالث عشر من الحمل، ثم تتكرر المعاملة مع المجموعتين الثانية والثالثة بنفس النظام والذبح.

أما المجموعة الثانية فقد عوملت بهرمون سيرم الفرسة الحامل (PMSG) وأيضاً بهرمون مشيمة المرأة الحامل بمعدل ٥ وحدات دولية لكل هرمون وبنفس النظام المتبع فى المجموعة الأولى.

أوضـــحت التحلـــيلات الإحصائية أن زيادة عدد مرات الحقن تؤدى إلى زيادة قيم الإختلالات المتحصل علـــيها فـــى خلايـــا الأجـــنة الـــناتجة مـــن كلا المعاملتين بهرمون FSH، كما وجد أن معدل الإختلالات الكروموسومية الناتجة من الحقنات المتتالية بهرمون FSH أقل من مثيلاتها المحقونة بهرمون PMSG.

ظهـــرت الفجـــوات الكروموسومية Chromosomal gap فى خلايا الأجنة بعد الحقنة الثالثة فى حالة المعاملة الأولى وبعد الحقنة الثانية والثالثة فى حالة المعاملة الثانية كما ظهر كذلك تضاعف كامل للمجموعة الكروموسومية Polyploidy بعد الحقنة الثالثة فى كل من المجموعتين تحت الدراسة.

أظهـــرت المعاملة بهرمون FSH نتائج أفضل من المعاملة بهرمون PMSG حيث أنها أدت إلى زيادة مواقع الإنغراس الجنينى الكلية كما يتأثر عدد ووزن الأجنة بمستوى معنوى بين المجموعتين.

ممـــا ســـبق يتضح أن المعاملة الهرمونية 5 I.U. FSH + 5 I.U. HCG أفضل من المعاملة الهرمونية 5 I.U. PMSG + 5 I.U. HCG حيث أنها أعطت أقل نسبة من التغيرات الكروموسومية وأعلى متوسطات لمواقع الإنغراس الجنينى ولم يكن هناك تغيراً معنوياً بالنسبة للأجنة الحية وعلية فإن هذه المعاملة مـــن الممكـــن أن تكـــون أفضل المعاملات الهرمونية إستخداماً فى إستحداث التبويض فى الفئران الصغيرة البيضاء كحيوانات تجريبية.