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EFFECT OF HORMONAL TREATMENTS AND CYTOGENETICAL ASPECTS ON OVARIAN FUNCTION OF EGYPTIAN BALADI CATTLE HEIFERS SUFFERING FROM GONADAL DYSGENESIS WITH SPECIAL REFERENCE TO SERUM PROGESTERONE LEVELS

By

M.S. HASSANANE*. A.S.S. ABDOON**, and KARIMA F. MAHROUS*

* Dept. Cell Biology, National Research Center, Dokki, Giza, Egypt.
** Dept. Anim. Reprod & A.I. National Research Center, Dokki, Giza, Egypt.

SUMMARY

Nine Egyptian Baladi cattle heifers 27-35 months of age, were divided into two groups. Group. 1, normal cyclic heifers, received no treatment (control). Group 2 heifers suffering from ovarian dysgenesis, never exhibited oestrus, were treated with Norgestomet ear implant +PMSG and $PGF_2\alpha$ for induction of oestrus. Blood samples were collected before and after treatment for determination of serum prgesterone levels using RIA. In addition, blood samples were collected from both groups for cytogenetical analysis. The data revealed that none of treated heifers showed symptoms of oestrus up to 10 days after treatment, rectal palpation on day 10 after the end of treatment indicated no ovarian cyclical changes (size or structure) which was also confirmed by the basal level of serum progesterone concentration (0.19 ± 0.02 ng/ml) on the same day. Moreover, cytogenetical analysis of 4 heifers (one had failed in blood culture) indicated a significantly higher ($P < 0.01$) numerical variations and chromosomal aberrations in autosomes and sex chromosomes of the treated heifers when compared with normal cyclic animals. In conclusion, the results indicated that gonadal development and response to exogenous gonadotrophin were affected by the chromosomal abnormalities in both autosomes and sex chromosomes in Egyptian Baladi cattle.

INTRODUCTION

During the last decade, great attention has been centered around the use of hormones in management of reproduction in bovine, one of these aspects is the hormonal treatments for induction of oestrus in prepubertal heifers.

Early studies indicated that late maturing heifers had a decreased production efficiency, because they did not cycle or they cycled late during their first breeding season (Lemeister et al. 1973). Such animals have infantile ovaries and never exhibit oestrus (Jainudeen and Hafez, 1980). However, age at puberty may be modified by genetic variables (Laster et al., 1972 and Brinks et al., 1978), levels of feeding (Deutscher, 1973) and by hormonal treatment such as progestogen (Gonzalez-Padilla et al., 1975., Sheffield and Ellicot, 1982) because the pituitary gland and ovaries can respond to exogenous hormones before puberty (Schams et al., 1981).

From the cytogenetical view, chromosome abnormalities involved with ovarian dysfunction were classified into four types, (1) monosomy X-chromosome (Huhtinen and Makinen, 1991) or

a mosaic chromosome complement of 59, X0/60, XX, in another case 59, X0/60, XX/61, XXX and in a third case 59, X0/60, XX/61, X0 mixoploid (Swartz and Vogt 1983), (2) X-chromosome trisomy (Lance et al., 1981), (3) structural aberrations involving the X-chromosome (El-Nahass et al., 1974) and (4) structural aberrations of the autosomal chromosomes (Jenkins et al., 1985).

The aim of the present investigation is to study the effect of hormonal treatment (Norgestomet + PMSG) on the ovarian function of Egyptian Baladi cattle heifers suffering from ovarian dysgenesis with special reference to serum progesterone concentrations, secondly, the relationship between chromosomal abnormalities and the ovarian function.

MATERIAL AND METHODS

Animals under investigation:-

Nine Egyptian Baladi cattle heifers 27-35 months age, raised in the experimental farm of Fac. Vet. Med. Suez Canal University, Ismailia, Egypt. Animals were Kept under routine feeding and management according to the governmental sytem.

Gynecological examiantion:-

The experiemental animals were divided into two groups according to the rectal examination findings. Group 1: (n=4) normal cyclic heifers with palpable corpora lutea on their ovaries, this group serves as a control. Group 2: (n=5) Baladi heifers of the same age (treated group), rectal palpation revealed the presence of infantiale ovaries .

Hormonal treatment:

Heifers in group 2, received Norgestomet implant (Syncro-Mate B, Intervet, Netherlands) containing 3 mg Norgestomet, 2 injection of 3 mg Norgestomet plus 5 oestradiol valerate were given at impla insertion. Implant remained in place for 9 da On day 7 of implantation 2000 I.U. PMS (Folligon, Intervet, the Netherlands) were injec i.m. At implant removal 25 mg $PGF_2\alpha$ analog Dinoprost (Lutalyse, Upjohn, U.S.A) was injec i.m. All treated heifers were monitored for oestr up to 10 days using an intact bull, rectal examined on day 10 after the end of treatment detect the ovarian cyclical changes.

Blood samples:-

10 ml of blood were collected into vacutainer tub by Jugular vein puncture. Blood samples wer collected before the start of treatment and 10 day after the end of treatment. Blood was centrifuge at 1500x g for 30 min. and serum was separate and stored at -20°C until progesterone assay.

Also, 10ml of blood were collected under asepti conditions using heparinized vacutainer tubes from all animals (control & treated heifers), thes samples were used for cytogenetical analysis.

Progesterone assay:-

Progesterone concentrations were assayed usin Coated. A. Count radioimmunoassay kits o Diagnostic Product Corporation (D.P.C. USA) according to the method adopted by Pratt et al. (1991). The sensitivity of the assay was 0.02 ng/ml, the intra and inter-assay coefficient were 11 and 7% respectively.

Cytogenetic analysis:-

Chromosome preparations were obtained from phytohemaglutinine (PHA) stimulated cattle lymphocytes that had been cultured for 72h. Two hours before harvesting the cells, colchicine (100ug/ml of medium) was added. Metaphase preparations were obtained using the standard techniques. After preparation the flame dried slides, 5% Giemsa stain were used for their staining then mounted with mounting media and examined after drying. For every animal, chromosomal analysis was carried out in 50 metaphase spreads. The results were statistically analysed using Chi-Square test according to Mather (1957). The Statistical analysis was carried out in two ways, the first is to detect the heterogenecity or the error among animals within each group, the second was the detection of significance between the control and treated animals.

RESULTS

Response to the treatment:

The present data showed that none of experimental heifers responded to the treatment with Norgestomet plus PMSG, or showed symptoms of oestrous signs during the period of observation. In addition, rectal palpation 10 days after the end of atment indicated no palpable corpus luteum or follicular development on the ovaries of the hormone treated animals.

Serum progesterone concentrations:

Results of serum progesterone levels indicated no significant change after treatment. Before the starting of treatment progesterone level averaged 0.16 ± 0.03 ng/ml (range from undetectable to 0.21 ng/ml) and it averaged 0.19 ± 0.02 ng/ml (range from 0.02 to 0.23 ng/ml) 10 days after the end of treatment.

Table 1: The results of the serum progesterone concentrations are showed in Table 1:

Animal No.	Before treatment	10 days after treatment
2	Non detectable	0.02
3	0.17	0.23
4	0.09	0.19
7	0.21	0.13
8	0.19	0.21
Mean ± S.E.	0.165 ± 0.026	0.156 ± 0.38

Cytogenetical analysis:

Cytogenetical analysis of the present study is presented in Table (2), and histogram 1. Moreover Fig. 2 and Fig. 3 showing some structural chromosome aberrations in the treated animals. It is clearly evident from the present data that the percentage of abnormal metaphases were significantly higher ($P < 0.01$) in hormonal treated animals than in the control heifers. Peridiploid was significantly ($P < 0.01$) higher in treated than in control heifers, that was reflected on the total numerical variations (significantly higher ($P < 0.05$) in treated heifers than control ones. Structural aberrations demonstrated that the gap involved autosomes, breaks (involved both autosomes and sex chromosomes), and total structural aberrations were significantly higher ($P < 0.01$) in treated heifers than control animals. In addition, the gap involved sex chromosomes was signficantly higher ($P < 0.05$) in treated heifers than in the control animals (Table 1).

Concerning the heterogenecity test between individuals in each group (error), no signifcant difference was noticed for any type of aberrations within the control group. Meanwhile the treated heifers group showed a significant ($P < 0.05$) difference in the numerical varition (peridiploid), which was reflected on the total numerical variations.

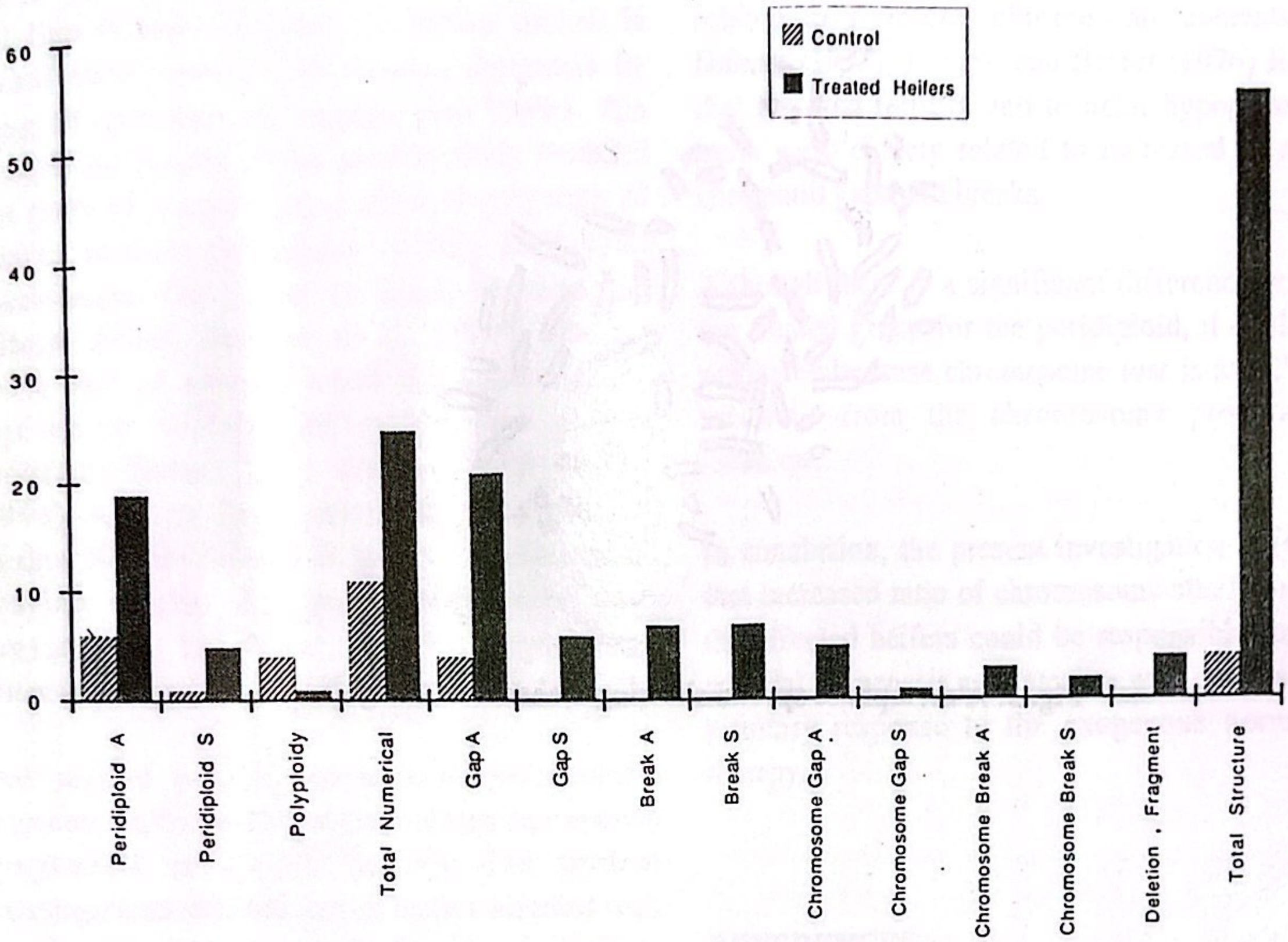
Table (٢) : Results of the cytogenetical analysis , Chi-square test within each group and Chi-square test between normal cycling females and the treated heifers.

		No. of animals	Total examined metaphases	Normal cells	Numerical variations				Structural aberrations									
					Peridiploid		Ploidy	Total	Gap		Break		Chromosome gap		Chromosome break		Deletion fragment	Total structure
					A	S			A	S	A	S	A	S	A	S		
Control	Total	4	200	183	6	1	4	11	4	0	0	0	0	0	0	0	0	4
	Error	—	—	2.469	2.06	3.014	2.041	4.135	2.041	0	0	0	0	0	0	0	0	2.041
Treated animals	Total	4	200	131	19	5	1	25	21	6	7	7	5	1	3	2	4	56
	Error	—	—	6.084	10.411*	8.82*	3.014	15.491**	1.861	11.684*	1.755	3.067	0.615	3.014	1.013	2.02	2.041	1.786
Chi-square difference		—	—	8.6**	7.76**	2.66	0.18	5.44*	11.56**	6*	7**	7**	2.5	0.5	3	2	2	45**

A : Autosome
S : Sex chromosome

* : $P < 0.05$
** : $P < 0.01$

Histogram 1: Different types of chromosome abevrations observed in the two studied group (Normal & Treated).



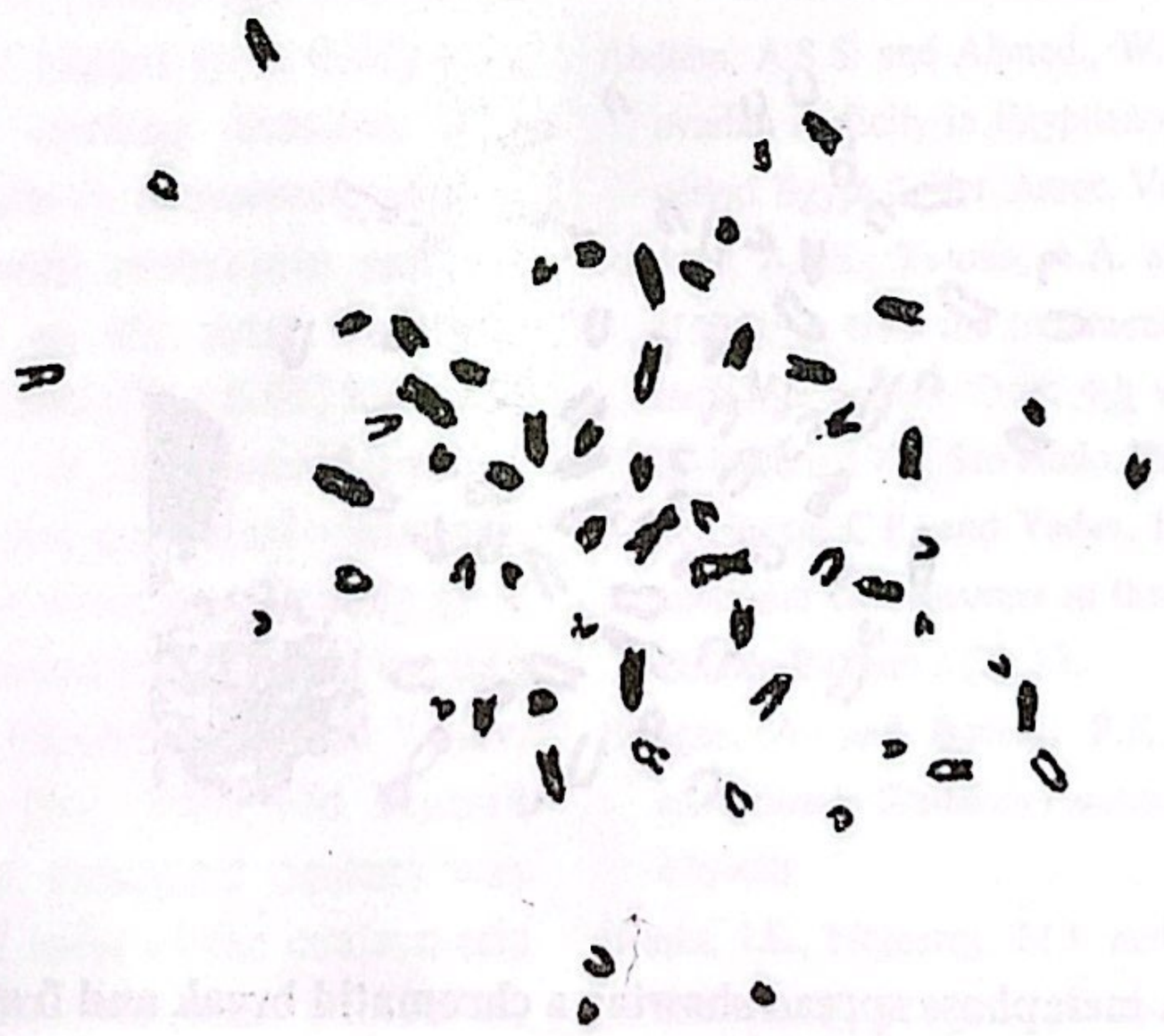


Fig. 1: Metaphase spread of a normal female cow.

Fig. 2: A metaphase spread showing a chromosome gap

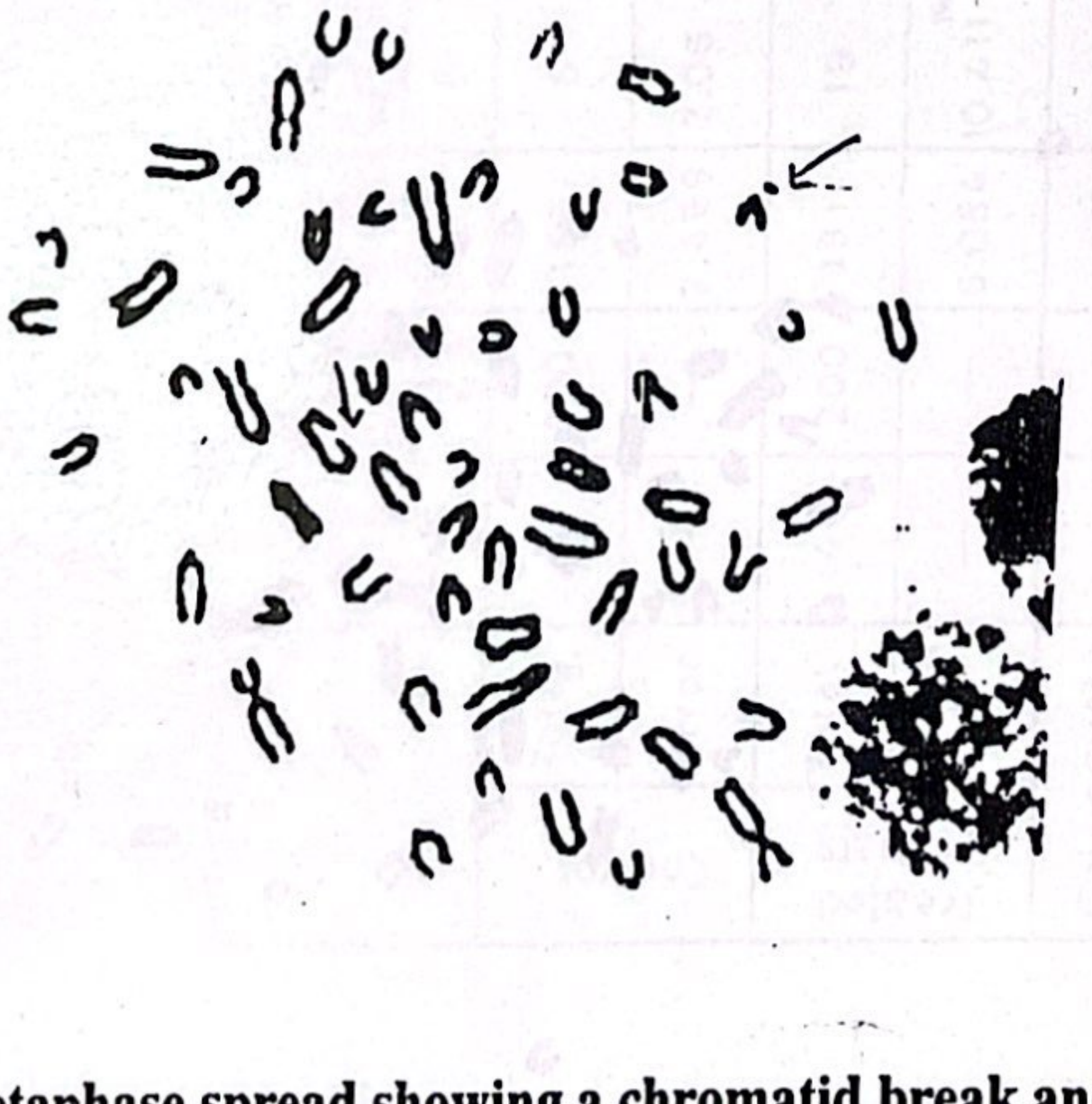


Fig. 3: A metaphase spread showing a chromatid break and fragment.

DISCUSSION

The results of the present study serve two aspects, ther first indicates a failure to induce oestrus in Baladi cattle heifers with ovarian dysgeneis by using Norgestomet ear implant plus PMSG. The unexpected results of the present study revealed that none of treated heifers showed symptoms of oestrus or male receptivity during 10 days of observation. On the other hand, Shefield and Ellicot (1982), Abdoon, et al., (1994) found a 100% rate of success when the same regimen applied to buffalo heifers, or post partum anoestrus Baladi cows (Abdoon and Ahmed (1994). Also, in the present study rectal palption on day 10 after the end of treatment indicated no ovarian cyclic changes which were also confirmend by basal serum progesterone concentrations (0.19 ng/ml) on the same day.

The second point deals with the relationship between chromosomal abnormalities and ovarian dysgenesis in Baladi heifers. The present investigations showed that in heifers affected with ovarian dysgenesis, while did not respond, to hormonal treatments, the percentage of aberrant cells were significantly higher ($p < 0.01$) than those in the normal cycling females. The percentage of peridiploids in autosomes, gaps in autosomes, break in both autosomes and sex chromosomes as well as the total structural aberrations were significantly($P < 0.01$) higher in affected heifers than it in the normal cycling females. Furthermore, total numerical variations, gap in sex chromosomes were significantly ($P < 0.5$) higher in treated heifers than normal cycling animals. In this respect, Balakrishnan, and Yadav, (1984) resported in a five years old Murrah buffalo cow that never exhibited oesturs was attribued to deletions of most of the centromeric regions of one of X-chromosomes. Moreover, autosomes can affect the development of gonads in human (Jenkins et a al. 1985) and Arabian filly (Trommershansen-Smith et al., 1979). Also the present results agree with Gustavsson (1980) who mentioned that lowered reproductive efficiency as a result of deficient ovarian development was related to structural chromosome aberrations. Halnan (1972), Bongso and Basrur (1976) found that lowered fertility and testiclar hypoplasia in bulls were closely related to increased rates of chromatid gaps and breaks.

Although there is a significant difference within the treated group for the peridiploid, it could be neglected because chromosome loss is usually an artifact from the chromosome preparation technique.

In conclusion, the present investigation revealed that increased ratio of chromosome aberrations in the affected heifers could be responsible for the gonadal dysgenesis and interfers with gonadal and pituitary response to the exogenous hormonal therapy.

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