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Reparative effects of lycium barbarum polysaccharide on mouse ovarian injuries induced by repeated superovulation

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ABSTRACT

To explore the repair effect of lycium barbarum polysaccharide (LBP) on ovarian injuries induced by repeated superovulation in mice, a model of ovarian injury was established, and ovarian repair was assessed after intragastric administration of LBP. The oocyte quality and blastocyst rates of pronuclear embryos *in vitro* were observed. The levels of 8-hydroxydeoxyguanosine (8-OHdG) and lipid peroxide (LPO) in ovarian tissue were measured, and ovarian damage was assessed in paraffin sections. The groups with significant injury were selected according to the above observation, mice in the significant injury group were intragastrically administered with LBP (low dose, 25 mg/kg; medium dose, 35 mg/kg; and high dose, 45 mg/kg) for 30 days. The above measurements and anti-Müllerian hormone (AMH) expression were detected in the mouse ovaries and the breeding verification was carried out. Our results showed that repeated superovulation could cause mouse oocyte quality to drop, significant differences started from 4 superovulation events ($P < 0.05$). The levels of 8-OHdG and LPO in the ovary increased gradually as the number of superovulation events increased, and significant differences were observed after 4–6 superovulations ($P < 0.05$). The ratios of primordial follicles, primary, tertiary and mature follicles decreased and the ratio of atresia follicles increased as the number of superovulation events increased, especially in 4–6 superovulation groups. Thus, the groups of superovulation 4–6 events were considered as significant injury groups. LBP-medium dose groups significantly improved the number and quantity of oocytes and embryo blastocyst rate ($P < 0.05$), significantly decreased 8-OHdG and LPO levels in mice ovary ($P < 0.05$), also improved the ratios of all stages follicles and reduced the rate of atresia follicles, increased the numbers of litter size, live birth, weaning survival, and repaired the expression of AMH in ovary significantly ($P < 0.05$). In conclusion, the degree of ovarian injury was affected by the number of superovulation. LBP repaired ovarian injuries most likely through scavenging oxidative products 8-OHdG and LPO and increasing AMH protein expression.

1. Introduction

With advancements in embryonic biotechnology and developmental biology research, the demand for oocytes and embryos is increasing. Superovulation via the injection of gonadotropin is an important method for obtaining more oocytes and embryos. The ovulation occurs similar to inflammatory reaction [1], which enhances the synthesis of prostaglandins [2], histamine [3], and bradykinin [4]. The ovulatory surge by gonadotropin induces an inflammatory reaction in mature follicles and that it is this inflammatory condition that brings about actual rupture of the ovarian surface [5]. Inflammation can cause oxidative stress, oxidative stress can also cause inflammation [6]. Oxidative stress products accumulate with the ovulation process, such as superoxide radicals , lipid peroxide (LPO) and 8-hydroxydeoxyguanosine (8-OHdG) etc. [1]. Study has shown that repeated superovulation by exogenous hormones can lead to oxidative damage to mitochondria and the production of oxidative stress products in mouse ovaries, including 8-OHdG and LPO [7]. These products can damage female genital organs [8] and change the concentration of ATP in oocytes, which results in decreasing oocyte quality and embryonic development ability [9]. Thus, during ovulation, oocytes and other cells are repeatedly subjected to oxidative stress, which may be the

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Table 1
The protocol for mouse superovulation (1–6 times).

Group	Mice (n)	First round		Second round		Third round		Fourth round		Fifth round		Sixth round	
		0 h	48 h	0 h	48 h	0 h	48 h	0 h	48 h	0 h	48 h	0 h	48 h
Control	12	saline	saline	saline	saline	saline	saline	saline	saline	saline	saline	saline	saline
1 time	12	saline	saline	saline	saline	saline	saline	saline	saline	saline	saline	PMSG	hCG
2 times	12	saline	saline	saline	saline	saline	saline	saline	saline	PMSG	hCG	PMSG	hCG
3 times	12	saline	saline	saline	saline	saline	saline	PMSG	hCG	PMSG	hCG	PMSG	hCG
4 times	12	saline	saline	saline	saline	PMSG	hCG	PMSG	hCG	PMSG	hCG	PMSG	hCG
5 times	12	saline	saline	PMSG	hCG	PMSG	hCG	PMSG	hCG	PMSG	hCG	PMSG	hCG
6 times	12	PMSG	hCG	PMSG	hCG	PMSG	hCG	PMSG	hCG	PMSG	hCG	PMSG	hCG

Control: superovulation 0 time group; 1 time: superovulation 1 time group.
2 times: superovulation 2 times group; 3 times: superovulation 3 times group; 4 times: superovulation 4 times group; 5 times: superovulation 5 times group: 6times: superovulation 6 times group.
The treatment of saline, PMSG, hCG was intraperitoneal injection.
Each round is divided by 5 days.

primary factor in ovarian aging. During ovarian aging, the organism produces a series of changes involving biological, physical and biochemical processes that result in cell and organ dysfunction and reduce oocyte quality [10]. At a certain stage of reproductive aging, considerable follicular atresia occurs in the ovary, and the follicle reserve decreases exponentially [11].

In recent years, Chinese herbal medicine has been widely used, Lycium barbarum is a typical medicinal and food plant with abundant resources. And its primary component, lycium barbarum polysaccharide (LBP), has functions on increasing immunity [12], anticancer [13], antioxidation [14], anti-aging [15] and reproductive protection [16–18]. Tian et al. found that polysaccharides could significantly increase the levels of three types of antioxidant enzymes and decrease level of malondialdehyde (MDA) in serum [19]. Yu et al. found that LBP has an antioxidant effect on cardiomyocytes of ovariectomy mice [20]. LBP can antagonize oxidative stress induced by diethylstilbestrol (DES) in adult male hamsters and alleviate hormone secretion disorder caused by DES [21]. Zhao et al. found that LBP can protect neurons from oxidative stress by increasing the activity of antioxidant enzymes and scavenging free radicals [22]. The antioxidation mechanism of LBP may be related to the inhibition of peroxidation induced by hydroxyl radicals, thereby protecting the biomembrane system from damage [23].

Some studies have shown that the traditional chinese medicine decoction containing LBP can improve the ovarian reserve ability of women [24,25], AMH is an important index to detect ovarian reserve capacity [26,27]. AMH can inhibit the growth of follicles to prevent the premature depletion of follicles, thereby ensuring an ovarian reserve [28]. AMH has an antifollicular atresia function, and intraperitoneal injection of AMH in mice can increase the number of oocytes in superovulation. AMH can regulate miRNA expression and further affect follicular growth hormone signal transduction and follicular development [29]. However, the effect of LBP on AMH in ovary is not clear.

To date, there have not seen any report on the role of LBP in repairing ovarian function damage caused by superovulation. Thus, in the present study, a mouse model of ovarian injury induced by repeated superovulation was established and administered LBP intragastrically to explore the repairing effect of LBP on ovarian damage and provide a new method for ameliorating ovarian injuries.

2. Materials and methods

2.1. Animals

Specific-pathogen-free (SPF) Kunming white female mice at 5 weeks old with a weight of 25 ± 1 g and SPF Kunming white male mice with a weight of 40 ± 2 g were obtained from the Chinese Academy of Military Sciences. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and were approved by the animal ethics committee of Beijing University of Agriculture and, and all efforts were made to minimize suffering. The experimental mice were allowed ad libitum access to food and water and raised in laboratory animal rooms with light and circulation control and subjected to a 14 h/10 h light/dark cycle.

2.2. Superovulation damage to ovaries and oocytes

2.2.1. Establishing a model of superovulation-induced ovarian injury

The mice in the experimental group were treated with injection of pregnant mare serum gonadotropin (PMSG) 10 IU (0.1 mL) at 18:00 and injection of human chorionic gonadotropin (hCG) 10IU (0.1 mL) 48 h later. The mice in the experimental groups were injected 1 to 6 times (6 groups), and at the same time, the mice in the control group were treated with saline injections (0.1 mL). Each group included 12 female mice. Superovulation rounds was first initiated in the superovulation 6 times group and the remaining groups were injected with saline, the next round began at 5 d after hCG injection of the previous round and so on, until the superovulation 1 time group was complete. The protocol for mouse superovulation (1–6 times) is shown in Table 1.

2.2.2. Collection and in vitro culture of oocytes and pronuclear embryos

Superovulation was induced in each group of mice according to the method above, and the mice (6 mice) were euthanized 26 h after hCG injection. The ovary and fallopian tube were removed, and the oocytes were obtained from the belly of the tubal ampulla and placed in PBS solution to observe and record the number and quality of oocytes. The obtained ovaries were washed three times with phosphate buffer saline (PBS) and then placed in a centrifuge tube in liquid nitrogen for the detection of 8-OHdG and LPO (left side of 6 mice in each group). Paraffin sections of ovarian tissue (right side of 6 mice in each group) were observed by histomorphology. The pronuclear embryos were obtained by injecting hCG into female mice (6 mice), and then introduced male mice in a 1:1 ratio, the mating success was confirmed by the presence of copulation plugs at 7:00 a.m.the next morning. The mice with copulation plugs were euthanized 26 h after hCG injection. The pronuclear embryos were obtained from the belly of the tubal ampulla, then granulosa cells were removed with 0.1% hyaluronidase and washed with PBS three times, then washed with modified

Table 2
The programme of LBP on the repair of superovulation injury.

Group		A	B	Mice (n)	Mice for oocytes(n)	Mice for pronucleus(n)	C (n)	D (n)	E (n)	Mice for mating (n)
NC		0 time	saline	18	6	6	6	6	6	6
4 times	DC	4 times	saline	18	6	6	6	–	–	6
	LBP-L		25 mg/kg	18	6	6	6	–	–	6
	LBP-M		35 mg/kg	18	6	6	6	–	–	6
	LBP-H		45 mg/kg	18	6	6	6	–	–	6
5 times	DC	5 times	saline	18	6	6	6	6	6	6
	LBP-L		25 mg/kg	18	6	6	6	6	6	6
	LBP-M		35 mg/kg	18	6	6	6	6	6	6
	LBP-H		45 mg/kg	18	6	6	6	6	6	6
6 times	DC	6 times	saline	18	6	6	6	–	–	6
	LBP-L		25 mg/kg	18	6	6	6	–	–	6
	LBP-M		35 mg/kg	18	6	6	6	–	–	6
	LBP-H		45 mg/kg	18	6	6	6	–	–	6

NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group.
4 times: superovulation 4 times; 5 times: superovulation 5 times; 6 times: superovulation 6 times; superovulations were performed according to Table 1.
The treatment of saline and LBP was fed intragastrically, each group was given intragastrically for 30 days.
A: superovulatory administration; B: intragastric administration; C: the number of ovaries for Detection of 8-OHdG and LPO; D: the number of ovaries for paraffin sections; E: the number of ovaries for AMH protein expression.

ChatotCL Ziomek CA and Bavister BD (mCZB) medium 3 times [30]. Then the embryos were covered with 50 μL mineral oil droplets in mCZB medium and cultured in a CO_2 incubator containing 95% air, 5% CO_2 and 100% humidity at 37 °C. The 2-cell, 4-cell, 8-cell, morula and blastocyst stages were observed at 24, 48, 72, 96, and 120 h respectively. At 48 h, the culture medium was replaced by mCZB with glucose.

2.2.3. Preparation of paraffin sections

The ovaries were washed 3 times with buffered saline (PBS), transferred into B's fixative solution (B1124, Applygen, China) and then washed three times, subsequently the ovaries were transferred to ethanol, dehydrated and embedded in paraffin. Serial sections with a thickness of 5 μm were stained with H&E, sealed with neutral gum, and each ovary had three slices, and we selected one slice from 15 ovarian tissue sections to observe ovarian organizational structure under 40x optical microscopy. The number of follicles at each stage and atretic follicles in the mouse ovaries were recorded.

2.2.4. Evaluations of oocytes and follicles

MII stage oocytes were divided into five grades according to the criteria of oocyte polar body integrity and the size of the peri-egg space: Class I: the first polar body is complete, the shape is round or oval, and the surface is smooth; Class II: normal perivitelline space, the first polar body is complete, the shape is round or oval, and the surface is crude; Class III: normal perivitelline space, and the first polar body is broken; Class IV: the perivitelline space is abnormally large, and the first polar body is giant; and Class V: the perivitelline space is abnormally large, and the first polar body is broken [31]. M I stage refers to oocytes that do not expel the first polar body.

The criteria for determining the follicle stages were as follows: follicle diameter, granulosa cell morphology and the number of layers around the oocytes. The follicles were divided into primordial follicles, primary follicles, secondary follicles, tertiary follicles, mature follicles [32]. Atresia follicles were characterized by collapse of follicle wall, irregular morphology of oocyte, nuclear condensation or dissolution. Granulosa cells and follicular membrane cells loose, atrophy and fall off into follicular cavity, zona pellucida collapse, etc. [33].

2.3. Detection of 8-OHdG and LPO

8-OHdG and LPO levels of the collected ovarian tissues were determined using an ELISA kit, according to the manu-facturer's instructions (Shanghai Hengyuan Biotechnology Co., Ltd, Shanghai, China). The collected mouse ovaries were cracked with lysate. Each 10 μL phenylmethane-sulfonyl (PMSF) was added in 1 mL radio immuno precipitation assay (RIPA) as lysate. Every 1 g of tissue with 9 mL lysate, then coated with mouse antibody against 8-OHdG or LPO. After incubation and washing concentrations of 8-OHdG or LPO were determined as described previously [34]. Intra-and inter-CVs: 8-OHdG (6.3%, 8.1%), LPO (5.2%, 7.4%).

Table 3
Effects of the number of superovulation events on mouse oocytes.

Group	No. of mice	Oocyte number (mean)	Oocyte ratio at all level (%) (Mean ± SD)						
			MI	I	II	III	IV	V	Fragment
Control	6	26.50 ± 2.38^{a}	13.96 ± 1.40^{a}	25.81 ± 2.33^{a}	26.57 ± 2.16^{a}	19.38 ± 2.67^{a}	6.81 ± 1.97^{a}	8.02 ± 2.24^{a}	0.45 ± 0.12^{a}
1 time	6	23.33 ± 2.56^{ab}	15.09 ± 1.53^{ab}	19.89 ± 1.59^{b}	22.68 ± 2.21^{ab}	19.75 ± 3.95^{a}	9.09 ± 1.46^{ab}	12.09 ± 2.01^{ab}	1.41 ± 0.67^{a}
2 times	6	19.67 ± 2.19^{bc}	17.65 ± 1.46^{ab}	12.76 ± 1.21^{bc}	17.65 ± 1.89^{b}	18.40 ± 3.01^{a}	11.76 ± 1.51^{b}	16.46 ± 2.31^{b}	5.32 ± 1.34^{b}
3 times	6	16.50 ± 2.13^{cd}	19.90 ± 2.08^{b}	8.51 ± 1.26^{cd}	14.76 ± 1.02^{c}	13.52 ± 2.56^{b}	17.05 ± 2.95^{c}	23.15 ± 3.59^{cd}	10.11 ± 1.89^{c}
4 times	6	15.17 ± 1.66^{de}	18.63 ± 2.07^{b}	6.43 ± 1.02^{de}	11.64 ± 1.11^{cd}	10.43 ± 2.06^{bc}	18.24 ± 2.77^{c}	28.30 ± 4.31^{d}	12.33 ± 2.17^{cd}
5 times	6	14.17 ± 1.96^{de}	17.82 ± 1.78^{ab}	5.56 ± 0.89^{de}	9.72 ± 0.76^{cd}	9.36 ± 1.98^{c}	20.20 ± 2.34^{c}	29.59 ± 4.35^{d}	12.75 ± 2.08^{cd}
6 times	6	13.17 ± 1.34^{e}	17.44 ± 1.94^{ab}	4.81 ± 0.98^{e}	7.86 ± 0.45^{d}	9.12 ± 1.79^{c}	21.55 ± 3.23^{c}	29.97 ± 4.81^{d}	13.25 ± 1.97^{d}

Control: superovulation 0 time group; 1time: superovulation 1 time group.
2 times: superovulation 2 times group; 3times: superovulation 3 times group; 4 times: superovulation 4 times group; 5 times: superovulation 5 times group; 6 times:superovulation 6 times group. MI: Immature oocytes.
I, II, III, IV and V are refer to MII oocytes: Class I、Class II、Class III、Class IV and Class V.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$. In the same column.

Table 4
Effects of the number of superovulation events on the *in vitro* development of mouse Pronuclear embryos.

Group	No.of mice	Pronuclear embryo number (each)	Unfertilized rate (%)	Development rate of embryos to stages (%) (Mean ± SD)				
				2-cells	4-cells	8-cells	Morula	Blastocyst
Control	6	24.00 ± 2.17^{a}	17.26 ± 1.57^{a}	88.74 ± 10.14^{a}	87.85 ± 8.60^{a}	82.72 ± 8.85^{a}	77.75 ± 6.54^{a}	67.18 ± 5.97^{a}
1 time	6	21.50 ± 1.82.^{ab}	18.26 ± 1.98^{a}	82.94 ± 9.26^{ab}	79.85 ± 7.77^{ab}	75.22 ± 9.32^{ab}	57.34 ± 7.06^{b}	48.68 ± 6.00^{b}
2 times	5	19.17 ± 1.45^{b}	19.77 ± 2.20^{a}	77.26 ± 8.48^{b}	63.84 ± 7.75^{b}	58.33 ± 7.73^{b}	35.12 ± 6.31^{c}	14.97 ± 2.68^{c}
3 times	5	17.00 ± 1.13^{c}	23.39 ± 2.13^{b}	72.23 ± 7.21^{b}	45.45 ± 8.16^{c}	22.18 ± 5.62^{c}	11.09 ± 2.28^{d}	5.27 ± 0.59^{d}
4 times	6	14.17 ± 1.06^{cd}	25.26 ± 2.20^{bc}	64.68 ± 9.28^{c}	41.58 ± 4.76^{c}	21.50 ± 4.60^{cd}	9.28 ± 2.30^{d}	0.00 ± 0.00^{e}
5 times	5	10.50 ± 1.72^{d}	26.71 ± 2.70^{c}	47.17 ± 8.24^{d}	23.26 ± 3.28^{d}	10.33 ± 1.55^{d}	1.22 ± 0.39^{e}	0.00 ± 0.00^{e}
6 times	5	7.80 ± 0.95^{d}	26.82 ± 3.16^{c}	42.14 ± 6.48^{d}	7.11 ± 0.83^{d}	0.00 ± 0.17^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

Control: superovulation 0 time group; 1time: superovulation 1 time group.
2 times: superovulation 2 times group; 3 times: superovulation 3 times group; 4 times: superovulation 4 times group; 5 times: superovulation 5 times group.
6times: superovulation 6 times group.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$. In the same column.

Table 5
Effects of the number of superovulation events on 8-OHdG and LPO levels in mouse ovaries.

Group	8-OHdG content (ng/L)	LPO content (pg/mL)
Control	83.51 ± 8.81^{a}	108.44 ± 10.18^{a}
1 time	91.70 ± 9.67^{a}	123.67 ± 11.21^{ab}
2 times	106.09 ± 11.01^{b}	131.86 ± 13.01^{bc}
3 times	112.22 ± 11.98^{b}	161.45 ± 15.45^{d}
4 times	119.90 ± 12.35^{c}	178.81 ± 16.01^{d}
5 times	136.15 ± 12.14^{cd}	217.11 ± 18.22^{e}
6 times	141.57 ± 15.27^{d}	227.94 ± 21.23^{e}

Control: superovulation 0 time group; 1 time: superovulation 1 time group.
2 times: superovulation 2 times group; 3 times: superovulation 3 times group; 4 times: superovulation 4 times group; 5 times: superovulation 5 times group; 6 times: superovulation 6 times group.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$. In the same column.

2.4. Repair effects of LBP on superovulation-induced injury

According to the above experiment, the significant injury superovulation 4–6 times were carried out according to Table 1, then administered LBP intragastrically at three doses (low, 25 mg/kg; medium, 35 mg/kg; and high, 45 mg/kg) as experimental group (18 mice each group), the saline was used as damage control group (18 mice), the superovulatory 0 time group was administered with saline as normal control group (18 mice). Mice were administered intragastrically at 10:00 a.m. every day for 30 d.

According to the above methods, oocytes (6 mice) and pronuclear embryos (6 mice) were collected and pronuclear embryos were cultured *in vitro*, the number of oocytes in each group and the development of pronuclear embryos *in vitro* were observed. And the concentrations of 8-OHdG and LPO in the ovaries were detected. Then, the mice (6 mice) were bred for verification. Finally, the ovaries of mice of superovulation 5 times each groups were selected for paraffin sectioning and histomorphological observation, and AMH protein expression was detected using western blots. The protocol of LBP on the repair of superovulation injury is shown in Table 2.

Western blots were performed using Automated Capillary Western Blot (WES) (ProteinSimple, San Jose CA) [35], an automated capillary-based size sorting system (ProteinSimple, San Jose CA). All procedures were performed with manufacturers reagents according to their user manual. Briefly, 8 μL of diluted protein lysate was mixed with 2 μL of 5× fluorescent master mix and heated at 95 °C for 5 min. The samples (1 μg), blocking reagent, wash buffer, primary antibodies, anti-AMH (Anti Rabbit, 1:100, ab103233, abcam, UK), secondary antibodies (Anti Rabbit, 1:500, 042-206SDS, Proteinsimple, San Jose CA), and chemiluminescent substrate were dispensed into designated wells in a manufacturer provided microplate. The plate was loaded into the instrument and protein was drawn into individual capillaries on a 25 capillary cassette provided by the manufacturer. Protein separation and immuno detection was performed automatically on the individual capillaries using default settings. The data was analyzed using Compass software (ProteinSimple, San Jose CA).

2.5. Statistical methods

All data are shown as the mean ± SD and were analyzed using SPSS 20.0 software. One-way ANOVA and Tukey's multiple comparison methods were used to determine statistical significance, Olympus DP-BSW 3.1 software was used to take ovarian slices picture, and Images advanced 3.2 software was used to count the number and ratio of follicles. The data in the table compares between the same column. A *P*-value less than 0.05 was considered significant, a P-value more than 0.05 was considered no significant difference.

3. Results

3.1. Superovulation damage to ovaries and oocytes

3.1.1. Effects of the number of superovulation times on the number and quality of mouse oocytes

As the number of superovulation events increased, the number of oocytes showed a downward trend, and the ratios of MI and fragmented oocytes increased gradually, whereas the ratios of MII showed different, such as class I, II, and III oocytes decreased, class IVand Vincreased. Compared with the control group and superovulation 1–2 times groups, MII oocyte numbers from superovulation 3–6 times groups showed significantly difference ($P < 0.05$), in which superovulation 4–6 times groups were more obvious (Table 3).

3.1.2. Effect of the number of superovulation events on the blastocyst rate of mouse pronuclear embryos in vitro

Our results showed that, as the number of superovulation times increased, the number of pronuclear embryos showed a downward trend, and the unfertilized rate increased. Beginning with the superovulation 2 times group, the 2-cell, 4-cell and 8-cell ratios decreased significantly ($P < 0.05$). The blastocyst rate of all superovulation groups was significantly lower than that of the control group ($P < 0.05$). As the number of superovulation times increased, the morula and blastocyst rates decreased significantly. In superovulation 4–6 times groups, 2-cell to 4-cell rate decreased obviously and failed to develop to blastocysts, which indicated that repeated superovulation affected embryo development quality, especially in 4–6 times groups (Table 4).

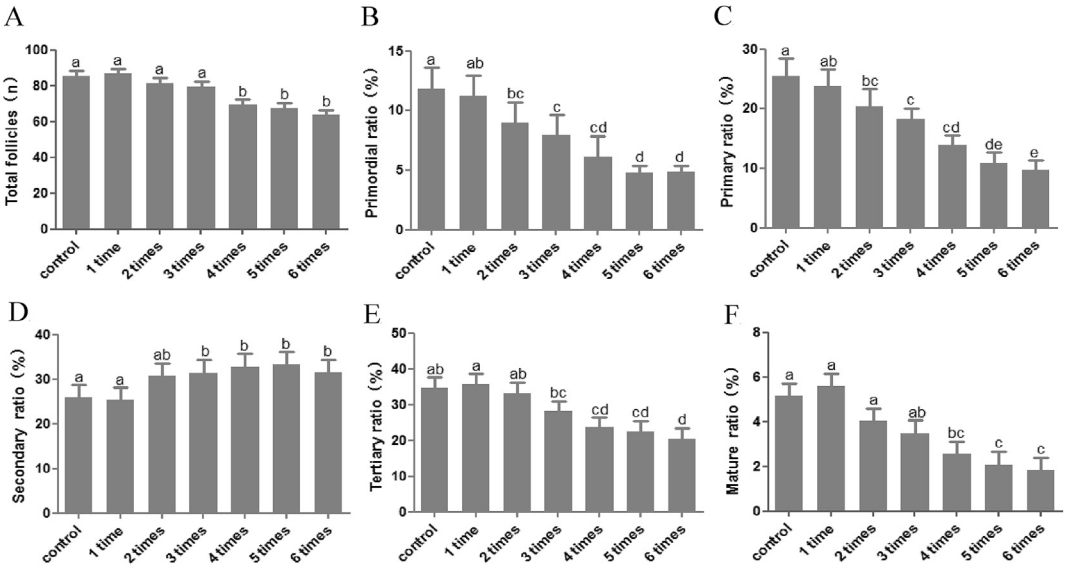

Fig. 1. Relationship between the follicular ratio and the number of superovulation events.
Control: superovulation 0 time group; 1 time: superovulation 1 time group; 2 times: superovulation 2 times group; 3times: superovulation 3 times group; 4 times:superovulation 4 times group; 5 times: superovulation 5 times group; 6 times: superovulation 6 times group. A: total follicles number; B: Primordial follicles ratio; C: Primary follicles ratio; D: Secondary follicles ratio; E: Tertiary follicles ratio; F: Mature follicles ratio. Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$.

3.1.3. Effects of the number of superovulation events on 8-OHdG and LPO in the mouse ovary

The contents of 8-OHdG and LPO in the ovaries were significantly higher than those in the control group starting from superovulation 2 times group ($P < 0.05$). As the number of superovulation events increased, the contents of 8-OHdG and LPO increased significantly. There was no significant difference between superovulation 5 times group and 6 times group ($P > 0.05$). (Table 5).

3.1.4. Relationship between the follicular ratio and the number of superovulation events

It can be seen from Fig. 1 that the total number of follicles, from the beginning of superovulation 4 times group, it was significantly lower than that of the control group, there was no significant difference in superovulation 4–6 times groups ($P > 0.05$). The ratios of primordial, primary, tertiary follicles and mature follicles decreased with the increase of the number of superovulation times, the decrease of superovulation 4–6 times groups was more significant.

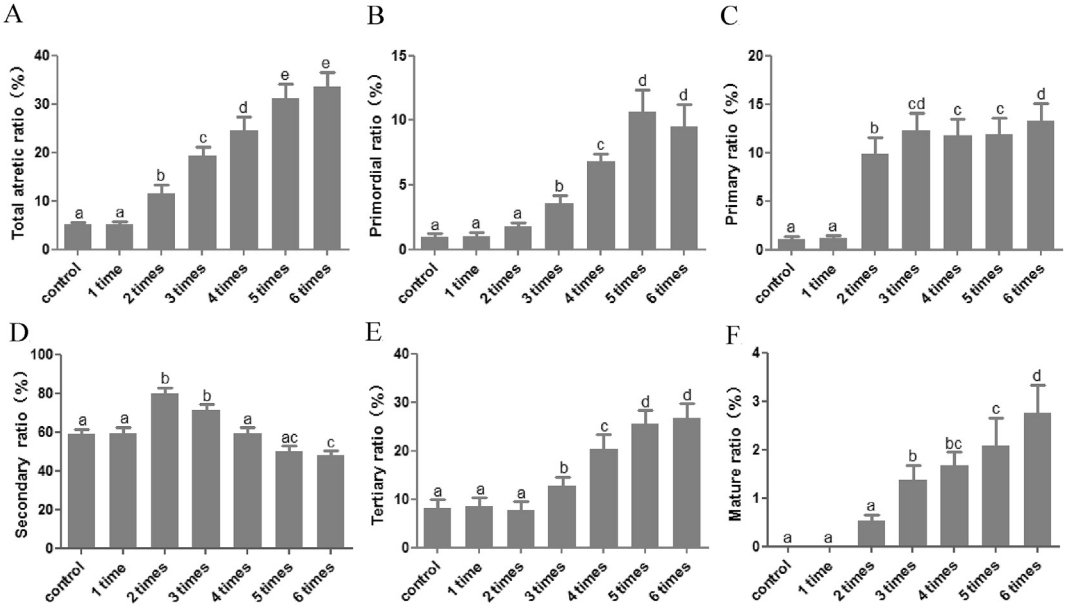

Fig. 2. Relationship between the follicular ratio and the number of superovulation events in atretic follicles.
Control: superovulation 0 time group; 1 time: superovulation 1 time group; 2 times: superovulation 2 times group; 3times: superovulation 3 times group; 4 times: superovulation 4 times group; 5 times: superovulation 5 times group; 6 times: superovulation 6 times group. A: total aretic follicles ratio; B: Primordial aretic follicles ratio; C: Primary aretic follicles ratio; D: Secondary aretic follicles ratio; E: Tertiary aretic follicles ratio; F: Mature aretic follicles ratio. Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$.

Table 6
Effects of LBP on oocyte repair in mice subjected to 4, 5 and 6 superovulation events.

Group		No.of mice	Oocyte number	Oocyte ratio at all level (%) (Mean ± SD)					
				MI	I	II	III	IV	V
NC		5	25.50 ± 2.03^{a}	13.96 ± 1.51^{a}	25.81 ± 2.79^{a}	26.57 ± 2.87^{a}	19.38 ± 2.09^{ab}	5.81 ± 0.63^{a}	8.02 ± 0.87^{a}
4 times	DC	6	16.67 ± 1.85^{b}	17.29 ± 1.87^{b}	4.76 ± 0.62^{b}	4.76 ± 0.78^{b}	9.52 ± 1.24^{c}	19.05 ± 2.49^{b}	33.33 ± 4.36^{b}
	LBP-L	6	21.08 ± 2.22^{c}	16.65 ± 2.16^{ab}	17.65 ± 2.16^{b}	11.76 ± 1.44^{c}	17.65 ± 2.16^{b}	15.65 ± 1.92^{c}	11.76 ± 1.44^{c}
	LBP-M	5	22.16 ± 2.47^{ac}	14.29 ± 1.81^{a}	23.81 ± 3.02^{a}	24.57 ± 3.11^{a}	19.05 ± 2.41^{ab}	6.76 ± 0.86^{a}	8.26 ± 1.02^{a}
	LBP-H	5	22.10 ± 2.16^{ac}	15.09 ± 2.15^{ab}	19.94 ± 2.31^{d}	21.18 ± 3.02^{d}	22.45 ± 3.20^{a}	9.09 ± 1.29^{a}	8.17 ± 1.05^{a}
5 times	DC	6	15.26 ± 1.32^{b}	18.38 ± 1.84^{b}	4.23 ± 0.51^{b}	4.46 ± 0.39^{b}	10.21 ± 1.23^{b}	19.72 ± 2.36^{b}	31.43 ± 3.77^{b}
	LBP-L	6	20.80 ± 2.08^{c}	17.92 ± 2.07^{bc}	15.65 ± 1.72^{c}	11.64 ± 1.28^{c}	18.33 ± 2.01^{a}	17.19 ± 1.88^{b}	12.48 ± 1.34^{c}
	LBP-M	5	21.33 ± 2.14^{c}	15.27 ± 1.86^{ac}	22.15 ± 2.69^{d}	24.05 ± 2.92^{a}	19.79 ± 2.40^{a}	7.42 ± 0.90^{ac}	9.10 ± 1.15^{a}
	LBP-H	5	21.31 ± 3.01^{c}	16.31 ± 2.10^{c}	19.09 ± 2.72^{e}	22.82 ± 2.68^{a}	21.95 ± 3.21^{a}	8.29 ± 1.19^{c}	9.19 ± 1.29^{a}
6 times	DC	6	14.83 ± 1.65^{b}	18.97 ± 1.98^{b}	4.01 ± 0.49^{b}	4.16 ± 0.53^{b}	11.23 ± 1.37^{c}	20.15 ± 2.46^{b}	32.33 ± 3.95^{b}
	LBP-L	6	17.98 ± 2.13^{c}	18.25 ± 2.51^{cb}	14.98 ± 1.96^{c}	11.03 ± 1.44^{c}	19.37 ± 2.53^{b}	18.05 ± 2.35^{b}	13.76 ± 1.54^{c}
	LBP-M	6	19.50 ± 2.76^{d}	15.95 ± 1.95^{ad}	21.81 ± 2.67^{d}	23.17 ± 2.84^{ad}	20.05 ± 2.46^{b}	7.86 ± 0.96^{ac}	9.78 ± 1.20^{a}
	LBP-H	6	19.16 ± 2.07^{d}	16.91 ± 2.12^{cd}	17.21 ± 2.16^{e}	21.12 ± 2.52^{d}	25.32 ± 3.17^{a}	9.98 ± 1.25^{c}	9.76 ± 1.17^{a}

NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group.
MI: Immature oocytes.
I, II, III, IV and V are refer to MII oocytes: Class I, Class II, Class III, Class IV and Class V.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$. In the same column.

In the ratio of secondary follicles, superovulation 3–6 times groups were significantly higher than control and superovulation 1 time group ($P < 0.05$), superovulation 2 times group had no difference with other groups ($P > 0.05$).

The ratios of total atretic follicles and the atretic ratio in primordial, primary, tertiary and mature follicles increased with the increase of the number of superovulation times, in which the increase of superovulation 4–6 times groups were more significant (Fig. 2). The ratios of mature atretic follicles in control group and superovulation 1 time group were 0. In the ratio of secondary atretic follicles, superovulation 2–3 times groups were higher than the other groups ($P < 0.05$), superovulation 6 times group was significantly lower than the other groups ($P < 0.05$) and had no difference with superovulation 5 times group ($P > 0.05$).

3.2. Repair effects of LBP on superovulation-induced injury

3.2.1. Repair effect of LBP on mouse oocytes

The results showed from Table 6, in the number of oocytes, superovulation 4–6 times damage control groups were significantly lower than normal control group, LBP-low, LBP-medium, LBP-high dose groups ($P < 0.05$), superovulation 4–6 times LBP-low dose groups were significantly lower than normal control group ($P < 0.05$), superovulation 4 times LBP-medium, -high dose groups had no difference with normal control group ($P > 0.05$), superovulation 5–6 times LBP-medium, -high dose groups were significantly lower than normal control group ($P < 0.05$), and had no difference between each other ($P > 0.05$). In the ratios of oocytes of MI and all classes of MII oocytes, superovulation 4–6 times damage control groups were significantly lower than normal control group ($P < 0.05$). In the ratio of MIoocytes, LBP-medium dose groups were significantly higher than damage control groups ($P < 0.05$), no difference than normal control group ($P > 0.05$). In the ratios of class I and II oocytes, LBP-medium dose groups were higher than damage control groups. In the ratios of class IV and V oocytes, LBP-medium dose groups were significantly lower than damage control groups and LBP-low dose groups ($P < 0.05$), had no significantly difference with normal control group and LBP-high dose groups ($P > 0.05$). The results showed that LBP-medium dose group had the best repair effect.

3.2.2. Effect of LBP on the blastocyst rate of pronuclear embryos in the superovulation-induced injury mouse model in vitro

In the number of pronuclear embryos (Table 7), superovulation 4–6 times damage control groups were significantly lower than normal control group, LBP-low, -medium, -high dose groups ($P < 0.05$), it was opposite that the number of unfertilized ratio was significantly higher than the other groups ($P < 0.05$). The number of

Table 7
Effects of LBP on the culture of mouse Pronuclear embryos *in vitro*.

Group		No.of mice	Pronuclear embryo number (each)	Unfertilized rate (%)	Development rate of embryos to stages (%) (Mean ± SD)				
					2-cells	4-cells	8-cells	Morula	Blastocyst
NC		5	21.2 ± 2.29^{a}	22.11 ± 1.90^{a}	86.17 ± 8.31^{a}	85.45 ± 9.23^{a}	80.36 ± 8.68^{a}	74.82 ± 8.08^{a}	62.31 ± 6.73^{a}
4 times	DC	6	12.22 ± 1.60^{b}	33.20 ± 2.27^{b}	70.21 ± 9.18^{b}	63.83 ± 8.34^{b}	52.75 ± 6.90^{b}	35.17 ± 4.60^{b}	21.69 ± 2.70^{b}
	LBP-L	5	16.40 ± 2.01^{c}	25.37 ± 2.46^{c}	73.66 ± 8.02^{bc}	68.84 ± 8.43^{bc}	63.15 ± 7.73^{c}	57.76 ± 5.97^{c}	42.71 ± 4.98^{c}
	LBP-M	5	20.45 ± 2.59^{a}	22.46 ± 1.92^{ac}	80.94 ± 9.26^{ac}	79.85 ± 10.12^{ac}	75.22 ± 9.53^{ac}	68.49 ± 8.17^{ac}	57.57 ± 7.30^{a}
	LBP-H	5	18.50 ± 2.64^{d}	23.05 ± 2.51^{c}	78.31 ± 10.16^{ac}	77.92 ± 10.83^{ac}	71.05 ± 10.12^{ac}	65.59 ± 9.20^{ac}	56.65 ± 8.07^{a}
5 times	DC	6	11.50 ± 1.38^{b}	32.81 ± 1.90^{b}	69.40 ± 8.32^{b}	62.61 ± 7.51^{b}	51.05 ± 6.12^{b}	31.28 ± 4.11^{b}	19.83 ± 2.38^{b}
	LBP-L	5	14.25 ± 1.56^{c}	29.00 ± 2.59^{c}	71.65 ± 7.86^{bc}	66.74 ± 7.32^{bc}	62.85 ± 6.89^{c}	55.69 ± 7.12^{c}	39.28 ± 4.31^{c}
	LBP-M	5	19.37 ± 2.35^{a}	23.47 ± 2.12^{ad}	78.42 ± 9.53^{ac}	77.82 ± 9.46^{ac}	73.91 ± 8.98^{ac}	67.50 ± 8.20^{ac}	52.26 ± 6.23^{d}
	LBP-H	5	17.45 ± 2.24^{d}	25.13 ± 2.37^{d}	76.31 ± 9.81^{ac}	77.01 ± 9.89^{ac}	70.72 ± 9.09^{ac}	63.21 ± 8.12^{ac}	49.95 ± 6.16^{d}
6 times	DC	6	11.03 ± 1.34^{b}	33.14 ± 2.76^{b}	68.21 ± 8.33^{b}	60.35 ± 7.37^{b}	49.35 ± 6.03^{b}	29.92 ± 6.14^{b}	16.92 ± 2.31^{b}
	LBP-L	5	13.90 ± 1.81^{c}	29.29 ± 2.62^{c}	70.36 ± 9.19^{bc}	64.94 ± 8.48^{bc}	61.81 ± 8.07^{c}	51.71 ± 9.97^{c}	32.37 ± 4.23^{c}
	LBP-M	5	19.02 ± 2.33^{a}	23.82 ± 2.16^{ad}	76.47 ± 9.37^{ac}	77.24 ± 9.47^{ac}	70.51 ± 8.85^{ac}	63.01 ± 9.21^{ad}	50.02 ± 6.25^{d}
	LBP-H	5	17.10 ± 2.14^{d}	25.38 ± 2.69^{d}	75.28 ± 9.43^{ac}	77.18 ± 9.67^{ac}	68.26 ± 8.68^{ac}	60.39 ± 8.82^{cd}	46.33 ± 5.81^{d}

NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$. In the same column.

Table 8
Effects of LBP on the levels of 8-OHdG and LPO in ovarian tissues of mice subjected to superovulation.

Group		8-OHdG content (ng/L)	LPO content (pg/mL)
NC		68.47 ± 8.68[a]	138.66 ± 16.92[a]
4 times	DC	115.66 ± 10.11[b]	217.71 ± 28.46[b]
	LBP-L	85.09 ± 8.97[c]	206.82 ± 29.46[b]
	LBP-M	70.66 ± 8.01[a]	132.10 ± 20.94[a]
	LBP-H	74.16 ± 7.26[ac]	147.70 ± 21.04[a]
5 times	DC	123.07 ± 11.25[b]	220.99 ± 25.78[b]
	LBP-L	112.60 ± 10.23[b]	206.94 ± 26.60[b]
	LBP-M	79.47 ± 7.34[ac]	136.71 ± 13.62[a]
	LBP-H	87.03 ± 8.12[c]	152.22 ± 16.20[a]
6 times	DC	131.53 ± 12.12[b]	231.19 ± 28.35[b]
	LBP-L	130.82 ± 12.97[b]	211.97 ± 26.56[b]
	LBP-M	109.15 ± 10.01[c]	165.25 ± 14.30[c]
	LBP-H	112.86 ± 11.22[c]	171.86 ± 19.87[c]

NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$.

Table 9
Effects of LBP on breeding and litter production in mice.

Group		Litter size	Live births	Weaning survivals	Offspring mice
NC		11.67 ± 1.13[a]	11.17 ± 1.01[a]	10.67 ± 1.45[a]	6
4 times	DC	9.20 ± 1.49[b]	8.60 ± 1.07[b]	4.80 ± 0.68[b]	5
	LBP-L	10.17 ± 1.34[b]	9.67 ± 1.38[c]	6.83 ± 0.86[c]	6
	LBP-M	11.50 ± 1.60[a]	10.83 ± 1.43[ac]	9.86 ± 1.20[a]	6
	LBP-H	11.03 ± 1.29[a]	10.63 ± 1.16[ac]	9.73 ± 1.11[a]	6
5 times	DC	8.25 ± 1.09[b]	6.50 ± 1.14[b]	3.75 ± 0.95[b]	4
	LBP-L	9.83 ± 1.16[c]	8.67 ± 0.81[a]	5.50 ± 0.64[c]	6
	LBP-M	10.33 ± 1.47[a]	9.83 ± 1.09[a]	8.67 ± 1.01[a]	6
	LBP-H	10.17 ± 1.33[a]	9.00 ± 1.11[a]	8.50 ± 1.07[a]	6
6 times	DC	7.95 ± 1.16[b]	6.15 ± 0.62[b]	3.50 ± 0.73[b]	4
	LBP-L	9.33 ± 1.21[a]	8.33 ± 1.20[a]	5.40 ± 0.69[bc]	5
	LBP-M	10.17 ± 1.24[a]	8.83 ± 1.14[a]	8.13 ± 1.47[a]	6
	LBP-H	9.50 ± 1.36[a]	8.60 ± 1.06[a]	7.97 ± 1.03[ac]	6

NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group.
Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$.

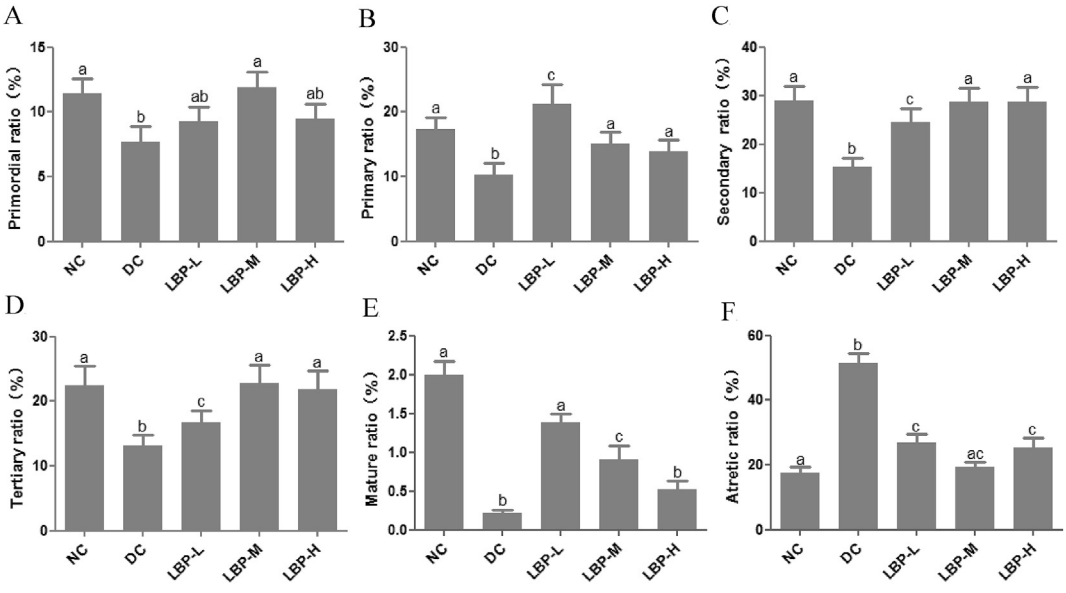

Fig. 3. Effects of LBP on ovarian follicles ratio in mice.
NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group. A: Primordial follicles ratio; B: Primary follicles ratio; C: Secondary follicles ratio; D: Tertiary follicles ratio; E: Mature follicles ratio; F: atretic follicles ratio. Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$.

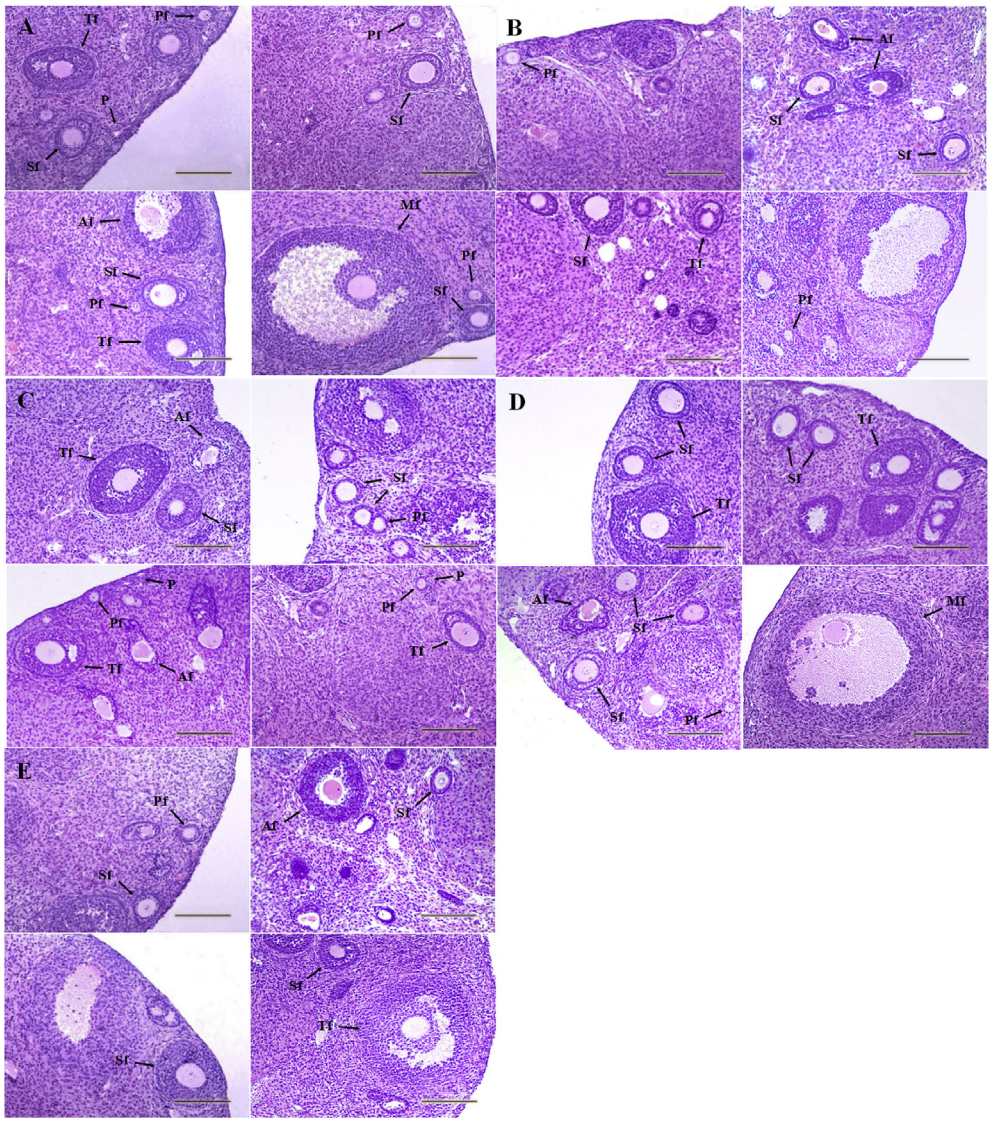

Fig. 4. Ovarian histology of mice from different groups.
A: normal control group; B: damage control group; C: LBP-low dose group; D: LBP-medium dose group; E: LBP-high dose group. P: primordial follicles; Pf: primary follicles; Sf: secondary follicles; Tf: tertiary follicles; Mf: mature follicles; Af: atretic follicles. Scale bar in figure is 200 μm.

pronuclear embryos in the LBP-medium group was significantly higher than that in the LBP-low and LBP-high groups ($P < 0.05$), which was not different from the normal control group ($P > 0.05$), while the unfertilized rate was opposite. In the embryonic development rates of each period (Table 7), the damage control groups of superovulation 4–6 times was significantly lower than the normal control group ($P < 0.05$), and the LBP-medium dose group was significantly higher than the injury control group ($P < 0.05$), which was obviously better than the other dose groups, there was no significant difference between normal control group except in the blastocyst rate ($P > 0.05$). In the blastocyst development rate, the LBP-medium group of superovulation 5–6 times was significantly lower than the normal control group ($P < 0.05$), but 4 times group was not significantly different from the normal control group ($P > 0.05$). It can be seen that the LBP medium dose group has the best repair effect.

3.2.3. Effects of LBP on the levels of 8-OHdG and LPO in ovarian tissue

Levels of 8-OHdG and LPO of superovulation 4–6 times damage control groups were significantly lower ($P < 0.05$) than normal control group, LBP medium-, high-dose group, and all damage control groups had no difference ($P > 0.05$) with LBP-low dose group except for 8-OHdG levels of superovulation 4 times. Superovulation 4–5 times LBP medium-dose groups were significantly lower than the other groups ($P < 0.05$), and had no difference with normal control group ($P > 0.05$) (Table 8). Superovulation 6 times groups were significantly lower than normal control group ($P < 0.05$).

3.2.4. Effects of LBP on breeding and litter production in mice

In the number of litter size, live births and weaning survivals (Table 9), superovulation 4–6 times damage control groups were significantly lower than normal control group, LBP-medium,-high dose groups ($P < 0.05$), LBP-medium and -high dose groups were better than LBP-low dose group, and had no difference between each other, LBP-medium groups were better than LBP-high dose group.

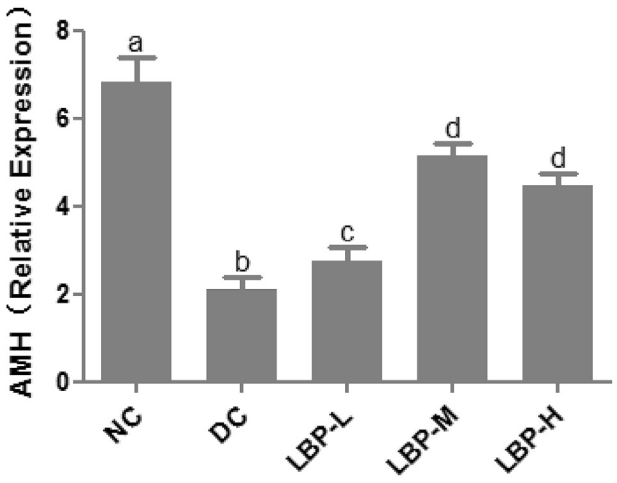

Fig. 5. Effect of LBP on AMH expression in the mouse ovary.
NC: normal control group; DC: damage control group; LBP-L: LBP-low dose group; LBP-M: LBP-medium dose group; LBP-H: LBP-high dose group. Different lowercase letters represent significant differences of $P < 0.05$, the same letters represent no significant differences of $P > 0.05$.

3.2.5. *Effects of LBP on the morphology of the mouse ovary*

Ratios of primordial follicles, primary follicles, secondary follicles, tertiary follicles and mature follicles of superovulation 5 times groups, damage control groups were significantly lower than normal control group ($P < 0.05$), and were significantly higher than normal control group in the ratio of atresia follicles ($P < 0.05$). LBP-low, -medium and -high dose groups were better than damage control groups in ratios of all stages follicles, and there was significant difference between LBP-medium group and damage control group ($P < 0.05$). LBP groups were significantly lower than damage control in atresia follicles ratios ($P < 0.05$) (Figs. 3 and 4).

3.3. *Effect of LBP on AMH expression in the mouse ovary*

In AMH protein expression level of superovulation 5 times groups (Fig. 5), damage control groups were significantly lower than normal control group, LBP-low, -medium, -high dose groups ($P < 0.05$). LBP-medium, -high dose groups were significantly higher than LBP-low dose groups ($P < 0.05$), significantly lower than normal control ($P < 0.05$), and there was no significant difference between each other ($P > 0.05$). LBP-medium dose groups were better than LBP-high dose groups.

4. Discussion

Our results showed that with the number of superovulation increase, the number of oocytes and pronuclear embryos decreased, the embryonic development rate of all stages decreased gradually, the ratios of atresia follicles increased, and the contents of peroxide 8-OHdG and LPO increased significantly. It is reported that 8-OHdG is a sensitive marker of DNA damage and can be used as a biomarker for oxidative stress injury [36]. LPO is produced by the reaction between oxygen free radicals and polyunsaturated fatty acids, under normal circumstances, LPO levels are very low, whereas under pathological conditions, increased lipid peroxidation can lead to increase LPO levels [37]. LPO and oxygen free radicals can destroy biofilm, ribonucleic acid and deoxyribonucleic acid, which are related to SOD and oxygen free radicals, which can inhibit immune function, and be related to tumor and to the production of some denatured proteins and can enhance platelet aggregation [38]. Liu et al. found that oxidative stress caused by high concentrations of ROS after ovulation decreased the developmental ability of aging oocytes [39]. The developmental ability of oocytes is related to the surrounding cumulus cells [40]. Cumulus cells provide energy substrate and essential nutrients to oocytes during oocyte development [41]. Xie et al. found that repeated superovulation affected mouse cumulus cells on mitochondrial function [42]. It is also reported that mammalian pre-implantation embryos cultured *in vitro* often fail to complete the whole development process from zygote to blastocyst in a medium of chemical composition determination, and stop at a particular stage of development, a phenomenon called development block [43]. *In vitro* development of mouse embryos was usually blocked in the 2-cell stage [44]. In this experiment, repeated superovulation increased the rate of atresia follicles and decreased the development rate of pronuclear embryos *in vitro*, and the ratios of 2-cell to 4-cell in 4–6 superovulation groups were significantly decreased, and no blastocysts were developed in the late stage, it indicates that as the number of superovulation increases, the lower the ability of the embryo to cross the embryo development block, the worse the quality of the embryo, which may be due to the increase of the accumulation of 8-OHdG and LPO in ovaries, thereafter affects the development and quality of follicles and oocytes. In addition, we also found that the atresia rates of secondary follicles did not increase with the increase in the number of superovulation as in other periods. The damage response of the secondary follicle to the superovulation was not as sensitive as the others periods follicle, so there was a tendency to be different from the other periods of the follicle. The related issues are needed to be further studied in the future.

Our results indicated that after LBP was intragastrically administered in mice damaged by repeated superovulation, the levels of 8-OHdG and LPO in ovaries and the ratio of atresia follicles were significantly decreased, and the quantity and quality of oocytes, the ratio of all stages follicles and the quality of pronuclear embryo were significantly improved. It is suggested that LBP can repair ovarian injury to a certain extent. When mice treated with LBP were bred, their litter size, live number and weanling survival number were significantly higher than those of mice with superovulation injury, it was verified that LBP could repair the injury of ovarian function and reproductive ability of mice caused by repeated superovulation, and LBP-medium dose has the best effect. It has been reported that LBP is a kind of plant polysaccharide with natural activity [45], it can be used to increase levels of superoxide dismutase (SOD) and glutathione (GSH) and so on, and remove excess lipid peroxide such as lactic dehydrogenase (LDH) and malondialdehyde (MDA), so as to enhance the antioxidant activity of cells and alleviate the oxidative damage of cells. Therefore, LBP can not only maintain the normal morphology and structure of cells and repair the morphology of damaged cells, but also inhibit the decrease of cell vitality and apoptosis induced by oxidative damage [46]. The results of this study suggest that LBP is able to reduce the oxidation products of 8-OHdGand LPO in ovaries, which may be beneficial to the improvement of ovarian function.

In this study, LBP significantly increased the ratios of primordial follicles and primary follicles, secondary follicles and antral follicles, decreased the ratio of atresia follicles in mouse ovaries, and significantly increased the expression of AMH protein in ovaries. It is reported that granulosa cells from the preantral follicles and small antral follicles secrete AMH, which inhibits the growth of follicles and prevents the premature consumption of follicles, thereby preserving the ovarian reserve, a positive correlation has been observed between AMH level and the number of antral follicles [47]. Study has shown that AMH can be used as an indicator of the ovarian response to controlled ovulation [48]. With the failure of primordial follicle development, the number of follicles

decreases, and the AMH level decreases, thus, AMH can be used as an index of ovarian reserve [49]. Lu et al. treated premature ovarian failure in rats using traditional Chinese medicine via umbilical curettage therapy and found that this therapy delays ovarian decline by upregulating the levels of vascular endothelial growth factor (VEGF) and AMH [50]. In addition, Zhang et al. showed that Guizhi Fuling capsules effectively improved the clinical features of patients with ovarian cyst after surgical removed the cyst nucleus by improving the level of AMH [51]. The results showed that LBP could repair ovarian follicular reserve and follicular development injury induced by repeated superovulation in mice.

5. Conclusions

Lycium barbarum polysaccharide can repair the ovarian damage caused by repeated superovulation, and improve ovarian reserve ability, oocyte quality and ovarian function, which may be attributed to reduce levels of 8-OHdG and LPO and increase AMH protein expression.

Author contribution

Bing Liu: Data curation, Formal analysis, Investigation, Software, Roles/Writing-original draft, Writing-review & editing, Validation. Jun-Li Wang: Data curation, Software, Investigation, Roles/Writing-original draft, Validation. Xin-Mei Wang: Conceptualization, Methodology, Investigation, Roles/Writing-original draf, Validation. Chao Zhang: Software, Investigation. Jia-Ge Dai: Investigation. Xiao-Meng Huang: Investigation. Jian-Ming Gao: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Roles/Writing-original draft, Writing-review & editing.

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